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Survey of Methods to Assess the Toxicological Impact of Hazardous Waste Disposal Sites on Aquatic Ecosystems

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SURVEY OF METHODS TO ASSESS THE TOXICOLOGICAL IMPACT OF HAZARDOUS WASTE DISPOSAL SITES ON AQUATIC ECOSYSTEMS

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This report provides an overview of toxicity testing methods useful for determining the impact of hazardous waste disposal sites on aquatic organisms and marine resources. These methods can be used in developing the information required by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) during the remedial investigation and feasibility study (RI/FS) process.

During the RI/FS process, data and information must be obtained to determine the nature and extent of hazardous waste impact to the environment. Methods described here will provide a basis for developing work plans and sampling protocols designed to address if and how coastal hazardous waste disposal sites impact aquatic resources.

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INTRODUCTION

This report provides an overview of toxicity testing methods useful for determining the impact of hazardous waste disposal sites on aquatic organisms and marine resources. These methods can be used in developing the information required by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) during the remedial investigation and feasibility study (RI/FS) process.

During the RI/FS process, data and information must be obtained to determine the nature and extent of hazardous waste impact to the environment. Methods described here will provide a basis for developing work plans and sampling protocols designed to address if and how coastal hazardous waste disposal sites impact aquatic resources.

TOXICOLOGICAL IMPACTS TO AQUATIC ORGANISMS

Each life stage of aquatic organisms has specific ecological requirements for survival and maturation. To make accurate toxicity assessments, the effects of toxins must be assessed at the life stage most vulnerable. Toxicity tests must be conducted under conditions which will simulate natural exposure to contaminants. Subtle, long-term chronic effects are difficult to distinguish from episodic, acute impacts, and from changes due to naturally occurring variations.

To determine the ecotoxicological impact, or the effect of pollutants on the environment and biota, four critical factors must be addressed: (1) identification of pollutants of concern and their sources and distributions; (2) determination of effects of these pollutants on species of concern at the single species level; (3) assessments of the effects of pollutants on populations and communities of organisms; and (4) determination of exposure and movement of contaminants through the ecosystem.

USE OF MICRO-ORGANISMS AND BIOCHEMICAL MARKERS FOR TOXICITY TESTING

Light-producing bacteria are used in a commercial bioassay system known as MICROTOX™. The procedure compares reduced light production from cultures of luminescent bacteria exposed to toxins to control cultures. Studies using the MICROTOX™ test system have obtained results comparable to other standard bioassay techniques, however, these results are not conclusive. MICROTOX™ offers reduced costs and ease of carrying out assays, however, the bacteria may not be as sensitive as other marine bioassay organisms and the results of differential toxicity are difficult to interpret.

Biochemical subcellular responses, or biomarkers, have been used to assess effects of pollutants. Hydrolases, metal-binding proteins and other stress-induced amino acids, and changes in the DNA of chromosomes offer promising possibilities for the determination of toxicological effects.

USE OF PHYTOPLANKTON IN TOXICITY TESTING

Phytoplankton, or algae, play an important role in all aquatic ecosystems. As primary producers, phytoplankton form the basis of the aquatic food chain and transfer energy to other trophic levels. Phytoplankton assays are simple to use, produce rapid

results, and are relatively inexpensive when compared to more complicated assays using fish and invertebrate species.

Algal species, such as dinoflagellates and diatoms, can be used to assess physiological effects of pollutants. Algal growth parameters can be measured using fluorescent techniques to determine inhibition from exposure to pollutants. These procedures provide quick results with minimal analytical requirements.

The Naval Ocean Systems Center (NOSC) is developing a bioassay procedure using luminescent dinoflagellate cells. This procedure measures reduced light output from the dinoflagellate cells in chronic exposures to low levels of contaminants. This bioassay is sensitive and able to detect various levels of toxicity at sublethal concentrations.

USE OF CRUSTACEANS IN TOXICITY TESTING

Many species of crustaceans have been used to test the toxicological impacts of heavy metals, pesticides, and other contaminants. The parameters most often measured in crustacean bioassays are percent survival, growth rates of populations, number of adults reaching reproductive age, number of larvae released by females, and numbers of progeny surviving. Many crustaceans such as the copepod *Acartia tonsa*, are widely distributed and play important ecological roles in aquatic ecosystems as herbivorous grazers and are important food items for larval fish.

Several species of crustaceans including amphipods and mysids have been used to assess the toxicological impact of contaminated sediments. Sediments are the final sink for many pollutants and the life history of these organisms are such that their close association with the sediments provides for realistic exposure to contaminants.

USE OF MOLLUSCS IN TOXICITY TESTING

The lifestyles and habitats of molluscs make them attractive for use in biomonitoring schemes. The bivalve mussel, *Mytilus edulis*, and species of the oyster, genus *Crassostrea*, are often used for pollution assessment. The molluscs are widely distributed, sedentary, have relatively high tolerance to pollutants, can be transplanted to areas of interest, and are able to integrate the effects of pollutants over long periods of time. The Mussel Watch Program, initiated by the U.S. Fish and Wildlife Service, uses mussels as sentinel organisms to monitor the coastal areas of the United States.

The initial emphasis of Mussel Watch was to test these organisms for tissue burdens of heavy metals, radionuclides, pesticides, hydrocarbons, and PCBs. Recently this program has increased its activities by measuring "scope for growth", the build up of stress enzymes, the presence of neoplasms, and pathological damage to tissue to provide sensitive early warnings of pollution impact. Other molluscan species are useful for toxicity assessments by virtue of their habitat and life history. The gastropod *Crepidula fornicata* and red abalone *Haliotis rufescens* show promise for use in short-term and chronic bioassays.

OTHER SPECIES TOXICITY TESTS: USE OF SEA URCHINS, MARINE WORMS, FISH, AND BIRDS IN TOXICITY TESTING

This section provides a quick overview of species for which toxicity testing is available and which might be required to assess impact at specific Navy hazardous waste sites. These tests include sea urchins, polychaetes, worms, fish, and bioassays using avian

populations. Sea urchins are ideal organisms because they are found along most coastlines and the biology of spawning, fertilization, and larval development has been defined for many species. Many of these bioassays examine the abnormal effects or delays in the routine development of eggs, sperm, and embryos for various species.

Polychaete annelids, or marine worms, have only recently been used in bioassays. The 96-hour LC_{50} test is commonly used because it is easy to conduct, results are rapidly obtained, and comparisons are feasible since many other bioassays are also conducted for 96 hours.

Fish species have been used historically to identify acutely toxic discharges. Acute tests use the 96-hour LC_{50} while chronic tests may last several weeks. The number of fish deaths is an end point in acute tests. Long-term, chronic tests can evaluate the toxicant's effect on behavior, growth, and reproduction. Toxicity tests on fish species are especially appropriate when contaminants may threaten important fish stocks and fisheries.

Many Navy hazardous waste sites are located in sensitive marshland and upland areas. Bird species like starlings, hawks, and herons are ideal for assessing impacts to wildlife. One avian bioassay uses starlings as bio-indicators of toxicological effects by establishing a quadrat of nest boxes to create a gradient of exposure to the hazardous waste site. Toxicological effects on survival, behavior, bioaccumulation, and reproductive success can be determined and evaluated for significant impacts by comparing results obtained along the gradient.

METHODS FOR MEASURING POLLUTION EFFECTS AT THE POPULATION AND COMMUNITY LEVEL

Pollution impact on an ecosystem will cause a shift in the resources available to organisms. This results in a change in the ability of the environment to provide the specific ecological requirements needed for a species to develop, mature, and produce offspring. Changes in the numbers of species, abundances, and shifts in the dominance of some species over others have been attributed to impacts from pollution. Changes in community structure and composition may provide powerful information on how contaminants impact aquatic ecosystems. It is difficult, however, to resolve the effects of pollutants from the natural spatial and temporal variability of the ecosystem. The use of diversity indices, monitoring indicator species, application of micro/mesocosms, construction of k-dominance curves, generation of Whittaker dominance-diversity curves, and the use of life tables and survivorship curves are evaluated as possible methods for determining pollution impacts at the population and community levels.

RECOMMENDATIONS

A combination of single and multispecies test procedures carried out in the laboratory and *in situ* are required to adequately assess the impact of contaminants on the surrounding ecosystem. Laboratory studies are useful for identifying the ranges or limits of impact which can be compared to field data. Multispecies testing will provide a range of sensitivities. The Navy should use existing data as a starting point for determining the optimum suite of tests that would be most applicable for detecting hazardous waste impact at a particular site. Once these tests and their results are compared, it may be practical to use only a few species for routine monitoring assuming the species selected accurately reflect the overall ecosystem response. A hypothetical example is used to illustrate how toxicity testing and biological assessment data can be integrated into the remedial investigation and feasibility study process.

INTRODUCTION

The impact of hazardous wastes on aquatic systems is an important consideration in the Navy's Installation Restoration Program (Little, 1987; Johnston *et al.*, 1988). A majority of the Navy's assets are located in coastal and estuarine areas which are complex and dynamic ecosystems, important both economically and ecologically.

Regulatory trends are moving toward more restrictive requirements with regard to marine pollution problems. The Comprehensive Environmental Response Compensation and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act (SARA), defines specific responsibilities of Federal facilities to clean up past hazardous waste disposal sites and requires clean up standards that are "protective of human health and the environment" (Public Law 99-499, Section 121.b, Oct. 17, 1986). Other federal laws such as the Clean Water Act (Public Law 100-4, Feb. 4, 1987) and the Resource Conservation and Recovery Act (Public Law 94-580, as amended by Public Law 99-499, Oct. 17, 1986) place increasing emphasis on regulating the impacts of hazardous substances on the environment. At the state and local level, the mandate for environmental protection is even stronger because the problems are closer to home. Elected officials and regulatory agencies are committed to enforcement of environmental laws and regulations.

The purpose of this report is to review methods available to assess the ecotoxicity of hazardous wastes in the marine environment and to provide input and guidance for determining the potential impact of hazardous materials and wastes at Navy facilities and adjacent aquatic sites. Ecotoxicity addresses the consequences of releasing pollutants in the environment and examining the effects on the biota that inhabit it (Butler, 1984). Ecotoxicity also addresses the final disposition of substances both biotically and abiotically once a substance is released into the environment.

While it is not practical to examine all toxic impact methods which have been described and used by various agencies, the more commonly used tools will be discussed.

TOXICOLOGICAL IMPACTS TO AQUATIC ORGANISMS

In the typical life cycle of marine invertebrates, eggs, and embryos are either released directly into the environment or are brooded by adults until prelarval forms develop before they are released into the environment (figure 1). Young planktonic larvae grow rapidly as they develop through various stages and change into juveniles. Juveniles develop into adults and produce gametes to repeat the cycle.

Each life stage requires different environmental conditions for survival and maturation. Pollution may effect an organism in different ways during its life cycle by increasing mortality (Epifanio, 1984; Green *et al.*, 1986; Laughlin *et al.*, 1978), reducing growth rates, (Epifanio, 1984; Green *et al.*, 1986; Davidson *et al.*, 1986), triggering endemic diseases (Dixon, 1982), and causing mutation in the development of gametes and embryos (Dixon, 1982; Nacci *et al.*, 1986; McGibbon and Molden, 1986). To make accurate assessments of toxicity on populations and communities of species it is very important to know which life stages are most sensitive to specific toxicants.

Larval stages of invertebrates are usually the most sensitive to pollutants (Epifanio, 1984; Laughlin *et al.*, 1978; Williams *et al.*, 1986; Green *et al.*, 1986). Larval toxicity is usually determined through the use of bioassays. Bioassays simply measure the toxicity of a particular substance under specific laboratory test conditions (Epifanio,

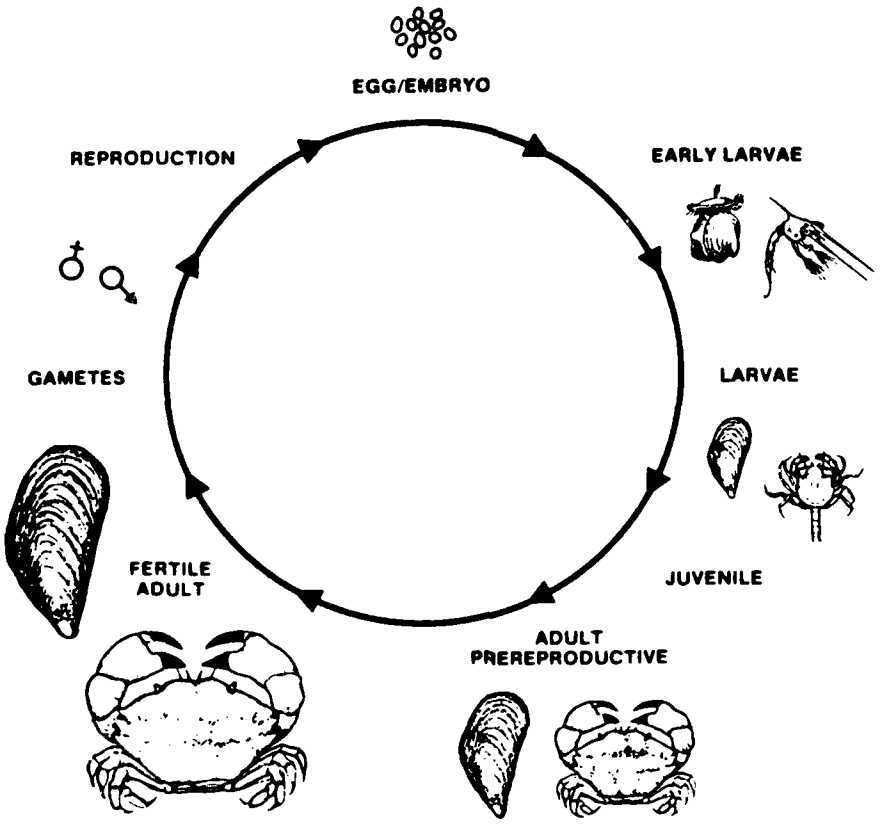

Figure 1. The generalized life cycles of marine invertebrates.

1984). Direct comparisons of bioassay results are limited to examining effects of varying concentrations of toxicant to the specific organisms tested (Epifanio, 1984), and often cannot be unequivocally related to field environmental conditions (Salazar, 1986). Bioassay-derived toxicity data are subject to wide variations due to age, sex, genotype, acclimatization of test organisms, and test duration (White and Champ, 1983). Rather than measure effects at realistic environmental levels (Epifanio, 1984), investigators usually assess high concentration effects and only infer what effects will be at lower concentrations.

For an assessment to be ecologically relevant, the effects of toxicants must be measured at several life stages of a test organism (Epifanio, 1984). An assessment must also account for natural conditions such as the toxin bioavailability (Salazar, 1986) and the acclimatization of test animals to environmental conditions (Beaumont and Budd, 1984). This assessment must be done to get even an "...incomplete idea of how pollution effects animals in [their] natural environment" (Epifanio, 1984).

Although eggs and embryos are relatively well protected, early larval stages of invertebrates seem to be the most effected due to their greater surface area to body weight ratio and direct exposure to waterborne toxins. LC_{50}s less than one-tenth of the adults have been reported for early larval stages (Green *et al.*, 1986; Williams *et al.*, 1986) as well as increased duration of larval stages (Epifanio, 1984; Laughlin *et al.*, 1978) which would add stress to the early larval stages already subjected to a high degree of

mortality. A 12- to 24-hour delay in molting could be fatal to larvae trying to avoid the intense predation pressure on planktonic organisms.

As larvae develop into juveniles, some species seek a suitable substrate on which to settle. The presence of pollutants may delay settlement of the organisms (Menzie, 1984), interfere with feeding activities (Robbins, 1985), impair food quality, and possibly alter sediment chemistry which could effect important chemical cues needed for recruitment (Menzie, 1984).

In the natural environment, pollutants may reduce primary productivity or cause a more nutritious algae to be replaced by a less nutritious species (Beaumont and Newman, 1986). Adult organisms can also be effected by abnormalities and morphological diseases that are endemic to populations which can be triggered by the additional stress due to pollution (Stephenson *et al.*, 1986; Paul and Davies, 1986). Adult mussels are also subjected to effects from the bioaccumulation of toxins even at relatively low concentrations (Goldberg *et al.*, 1978; Amiard-Triquet *et al.*, 1986; Holwerda and Herwig, 1986). Toxins are usually concentrated in specific organs such as the liver and gonads (Amiard-Triquet *et al.*, 1986) and have caused abnormal development of gametes and aneuploidy in embryos (Dixon, 1982; McGibbon and Molden, 1986; Nacci *et al.*, 1986; Gibbs and Bryan, 1986). Some species may also respond to pollution effects by increasing their abundance. For example, pollution causing eutrophication has been shown to stimulate the polychaete *Streblospio benedicti* to produce brood sizes 2 to 5 times greater than normal (Levin, 1986). Under these conditions, *S. benedicti* could completely dominate the soft-bottom community.

Acute (lethal, short term exposure tests, 24 to 96 hours) and chronic (sublethal, long term exposure tests of weeks to months) methodologies have been used in the laboratory and the field to measure physiological, reproductive, and behavioral effects on many species of marine plants and animals. Single species, multispecies, and assemblages of organisms have been the target of studies to elucidate what may happen to these organisms at the actual site (field validation). Background data for a specific site must include the toxic substances present and the route of dispersion into the site. Biological and chemical sensors can be used to measure and define the impact on biological communities. Impact can be defined as the effect the toxic substance(s) has on (1) life history strategy (benthic dwelling, sedentary, pelagic, filter feeding, detritovore, and omnivore), (2) bioconcentration of the contaminants in tissues which reflects the exposure levels in the ecosystem, and (3) survival determined by a series of acute tests with single species, chronic assessments with single and multiple species, and/or species assemblage mesocosm studies. In most cases, the test will be indicative of the environmental insult, under either lethal or sublethal conditions.

In assessing ecotoxicity of the impact of hazardous wastes on the marine environment, one must first inventory sources and routes of pollutants. Some of this information may be available from studies conducted at the site. However, the routes of dispersion may not be obvious and may require substantial investigative effort. In many cases, contaminant routes may be through runoff and ground water vectors. Toxic wastes usually consist of a composite of many toxicants forming complex mixtures which may be more toxic than single contaminants.

To conduct an ecotoxicological evaluation, an awareness of several critical parameters is essential. One must first try to identify the pollutants and in what parts of the ecosystem they occur. Second, one should try to understand the effects of the pollutant/pollutants on individuals or a few species, particularly on physiological processes. Third, one must study the pollutant's effects on communities of organisms

in situ, and/or in the laboratory to identify the sensitive species which could be used as indicator species. Lastly, one should determine quantitatively the movement of pollutants through the ecosystem, including amounts reaching the target species via water and soil (Butler, 1984).

Single species tests conducted in the laboratory usually consist of acute exposures resulting in death of the test organism (LC_{50}). Such studies have been conducted with algae, crustaceans, molluscs, echinoderms, polychaetes, and fish. It is usually possible to determine if there are impacts of immediate concern by conducting short-term tests, however, it is often necessary to conduct tests to determine the potential impact from long-term exposure. Appropriate long-term tests can identify potential problems which can be avoided by effective management strategies. The ability to conduct long-term bioassays on any species requires detailed knowledge of the proper maintenance requirements of the organism in the laboratory, observation of subtle behavioral effects, documentation of reproductive success, and measurement of growth rates (biological endpoints). Although long-term chronic studies can provide data concerning an organism's response to a range of dosing levels, these studies can be resource intensive.

Much work has centered on single species testing to determine what may occur in the real world under exposure to pollutants in the marine environment. The technique of multispecies testing provides a more realistic awareness of which organisms could be effected at various trophic levels and how biological structure is altered within aquatic communities. Multispecies testing may be more efficient than conducting several single-species tests because of the greater range of sensitivities afforded by many test organisms (Giesy, 1985). The use of multiple species may require more effort for maintenance of the population (Henderson, 1985; Valkirs *et al.*, 1985), but this effort may be justified by the range of responses exhibited by the different species. The debate among marine scientists in using single species versus multispecies testing is ongoing and will presumably continue until more multispecies testing produces enough data for comparison (Birge and Black, 1985). While single species tests are probably adequate to determine the immediate severity of contamination, the data obtained from multispecies tests will provide the information necessary to forecast potential long-term impact and provide the lead time necessary to develop management options to reduce or eliminate harmful exposure.

USE OF MICROORGANISMS IN TOXICITY TESTING

Some early observations indicated the presence of various toxins would reduce the light intensity from cultures of luminescent bacteria (Beijerinck, 1889). Since then, the utility of luminescent bacteria has been investigated for testing air quality (Sie *et al.*, 1966), herbicides (Tchan and Chiou, 1977), and other toxic chemicals (Johnson *et al.*, 1942). One study showed pronounced light inhibition by chlorides of copper, cobalt, manganese, nickel, and silver. Luminescent bacteria (*Photobacterium phosphoreum*) have been used in conjunction with the green alga *Chlorella* as a two-organism coupled assay (Tchan *et al.*, 1975). This technique is based on the ability of a herbicide to interfere with the photosynthetic activity and oxygen production of *Chlorella*. Oxygen levels are quantified using a culture of *Photobacterium* which luminesce at a rate proportional to oxygen produced. This technique was later modified by using the alga *Dunaliella tertiolecta* with *P. phosphoreum*. The assay was demonstrated to be sensitive to herbicide concentrations ranging from 0.001 to 4.0 mg/l (Tchan and Chiou, 1977).

The MICROTOX™ bioassay (Bulich, 1979) uses freeze-dried preparations of *Photobacterium phosphoreum* which are reconstituted with distilled water and chilled. This procedure then compares reduced light production from the cultures of luminescent bacteria exposed to toxins to control cultures.

Improvements in the original MICROTOX™ design have been implemented (Bulich *et al.*, 1981) with best results obtained when percent light decrease is expressed as a gamma function of light extinction (Johnson *et al.*, 1974). A MICROTOX™ correction procedure has also been developed for testing the toxicity of turbid aqueous samples. Reproducibility of the MICROTOX™ test results are varied but the coefficient of variation in light output for pure chemicals (Curtis *et al.*, 1982; Qureshi *et al.*, 1982), complex effluents (Qureshi *et al.*, 1982), waste treatment (Casseri *et al.*, 1983), and waste drilling fluids (Strosher, 1984) ranged from approximately 10 to 30 percent.

The MICROTOX™ test has been compared with other bioassay tests using fish (fathead minnows) and *Daphnia* (a small freshwater crustacean). In one particular study, the MICROTOX™ EC_{50} (50 percent light reduction observed) test sensitivity was comparable with the rainbow trout (96-hour LC_{50}), and *Daphnia* 48-hour LC_{50} bioassays, particularly for organic compounds and complex effluents (Qureshi *et al.*, 1982). The MICROTOX™ bioassay has been used for assessing the toxicity of contaminated marine sediments. Significant correlations between acute toxicity expressed as 15-minute EC_{50} and concentrations of sums of measured aromatic hydrocarbons, napthalenes, and chlorinated hydrocarbons have been demonstrated (Schiewe *et al.*, 1985).

The advantages of using MICROTOX™ versus the other standard bioassays are capital and operational costs are moderate, the time needed to conduct the test is on the order of 30 minutes to a few hours, the system is simple to operate, and reproducibility is good. However, this assay may not be as sensitive a technique as other assays and is not yet recognized for use by the Environmental Protection Agency.

Assays have been developed that measure motility of a cell in response to a toxicant. A motility test developed for the aquatic bacterium, *Spirillum volutans*, is based on observing a decrease in reversing motility of 90 percent of the test cells. The toxic effect is reported as the concentration of toxicant required to cause 90 percent inhibition in a set period of time (Trevors, 1986). This assay has been used in conjunction with other assays to determine the toxicity of pure compounds and complex effluents with varying degrees of success (Qureshi *et al.*, 1982). Toxic effects of heavy metal mixtures on *S. volutans* have been observed (Dutka and Kwan, 1982) using these methods.

Biochemical effects (molecular level effects) as indicators of prelethal or sub-lethal stress are being investigated at Naval Ocean Systems Center (NOSC). Hydrolases such as lysozymes in the serum of mussels, oysters, or clams may indicate cell membrane breakdown by metals (Pickwell and Steinert, 1984; Steinert and Pickwell, 1984). Elevated levels of metal binding proteins in the serum indicate metal uptake corresponding with elevated exposure to metals in the environment. General stress indicators such as heat shock proteins can be elevated in the cellular fluids by increased heavy metal exposure (Steinert and Pickwell, 1988).

THE USE OF PHYTOPLANKTON IN TOXICITY TESTING

Various phytoplankton species have been used as biological tools in assessing environmental impact. Their use in bioassays is justified by the ecological role they play as primary producers with respect to other trophic levels. Phytoplankton are relatively simple to use and inexpensive when compared to more complicated and involved assays using fish and invertebrate species (Wong and Couture, 1986). Their use in bioassays has become widespread internationally because phytoplankton are sensitive indicators of stimulatory or inhibitory effects of environmental pollutants. In general, there are two avenues for using phytoplankton as indicators of toxicity in the environment: continuous culture techniques in the laboratory (batch) and field methods.

In batch culture, phytoplankton is cultured, exposed to a toxicant, and observed over a period of time (usually during log phase of growth). This technique has been widely used and has been proposed as a "standard method" of phytoplankton bioassay. Batch culture systems, however, often contain artificially high nutrient concentrations which are atypical of natural environments.

Two types of systems are commonly used to maintain algal populations for toxicity testing, a turbidostat-controlled system and a chemostat-controlled system. The turbidostat is a device which maintains a constant algal cell number, a fixed ratio of cell number and toxicant, and a nutrient level similar to that existing in natural waters. The turbidostat uses measurements of turbidity using a photodetector to maintain the fixed ratio of cells. The disadvantages of using the turbidostat system are equipment setup time, adjustment and maintainance of photodetector sensitivity, high commercial cost of the turbidostat unit, and difficulties with microbial contamination of the culture vessel.

The chemostat system controls cell density in cultures by regulating the rates and concentrations of added nutrients. Increased rates of nutrients added to the culture medium will increase cell growth rates. Chemostats have been used extensively in the study of nutrient limitation on algal growth (Rhee, 1980; Droop, 1969), toxicity of PCBs (Fisher *et al.*, 1974), and mixtures of metals on phytoplankton. The chemostat method appears to be in use more than the turbidostat technique, possibly because it is easier to use. However, problems may be encountered maintaining constant flow rates with peristaltic pumps required by the chemostat-controlled system (Wong *et al.*, 1983).

While the above procedures pertain to the monoculture of a specific plankton species in the laboratory, perhaps a more realistic evaluation of the impact from hazardous substances at an aquatic site is the observation of an "effect" on the natural assemblage of species present at the site. Extrapolation from a single species effect to a community effect is tenuous, at best. In fact, the community response of an indigenous population may be a more sensitive indicator as some chemicals could cause a shift in the local species assemblage and dominant organisms attributable to the tolerances of different species (Cairns, 1980; Menzel and Case, 1977). Biologists must devote more energies in developing methodologies to assess aquatic community responses. The advantages of using community algal bioassays over single algal tests are (1) reduced costs by testing a greater number of species simultaneously; (2) realistic results obtained by testing indigenous species responses to particular toxic substances; (3) less time and expense required to carry out the test with relatively inexpensive equipment; and (4) a more complete understanding of the effect of chemicals to natural ecosystems. (Cairns, 1980).

To observe direct effects on the community, water samples can be collected, phytoplankton species identified, and biomass quantified. These samples can be

collected from nets, bottles, or pumped water from predetermined depths. Several studies have indicated that increased levels of zinc in streams and rivers decreased the number of phytoplankton species normally found (Whitton and Diaz, 1980), but since blue-green algae are more resistant to zinc, they are often numerous in waters with high levels of metal (Shehata and Whitton, 1981). Other studies have documented the shift in species diversity and dominance within phytoplankton systems impacted by heavy metals (Cote, 1983; Thomas and Seibert, 1977; Saward *et al.*, 1975). Chemical stress on algal communities reduces the number of species present, increases the numbers of individuals per species, and shifts the dominance within a community, favoring some species over others (Cairns and Lanza, 1972; Krett, 1979).

There are several methods for measuring stress in algal communities. One of the most widely used methods is making phytoplankton cell counts. The effect of the toxicant is determined by enumerating cell numbers during the assay and comparing to control cell numbers. Detailed procedures for screening toxicity of chemicals on phytoplankton have been proposed by the International Organization for Standardization (Stratton *et al.*, 1980) and, although developed for freshwater algae, these procedures could probably be used for marine phytoplankton until other test standards are completely developed for marine phytoplankton. In principle, algal growth media containing the toxicant is innoculated with a small number of phytoplankton cells. The population is assayed at intervals by cell counts and compared to control cell counts incubated under identical conditions. Cell growth is measured for a period of at least 3 days or up to a time when cell growth has reached the stationary phase (Wong and Couture, 1986). For example, figure 2 shows the effect of various concentrations of potassium dichromate on the growth of the alga *Scenedesmus subspicatus*. Cultures of marine phytoplankton are available from various institutions in the country (i.e., Center for the Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, McKown Point, West Boothbay Harbor, Maine 04575).

Marine luminescent dinoflagellates are currently being used to detect the toxicity of various environmental contaminants. A significant reduction in light output has been measured from the nonthecate dinoflagellate *Pyrocystis lunula* when cells of this species were exposed to 2 μg/ml (ppm) of T-2 trichothecenes (yellow rain compounds). These compounds have been observed to quench the luminescence of test cultures by as much as 40 percent following 2 hours of exposure (Hannan *et al.*, 1986). They also observed a substantial difference between the response of *P. lunula* and a luminescent bacteria isolated from the Gulf of Mexico. While quenching of luminescence in *Pyrocystis* was observed at less than 1.0 μg/ml, no quenching of bioluminescence was observed from the bacteria at 10 μg/ml of T-2 trichothecenes. In a more recent study, we observed two effects on the light output from cells of the same dinoflagellate exposed to low levels of tributyltin chloride (TBTCl). At levels of exposure from 0.2 to 2.2 μg/l (ppb) TBTCl, the decrease in light output ranged from 30 to 46 percent. An even greater reduction in light output (80 to 99 percent) was observed from cells cultured in 3.7 to 29 ppb TBTCl media. The use of this assay may provide a highly sensitive test because acute as well as sublethal physiological effects can be quickly measured by observing bioluminescence from hours to days following exposure to toxicants.

Another technique for assessing the physiological state of growing algal populations is observing changes in chlorophyll *a* fluorescence. The level of chlorophyll fluorescence is directly related to phytoplankton cell concentrations (Samuelsson *et al.*, 1978). Algal growth media containing a toxicant is innoculated with a small number of cells and the physiological state of the growing cell population is measured following

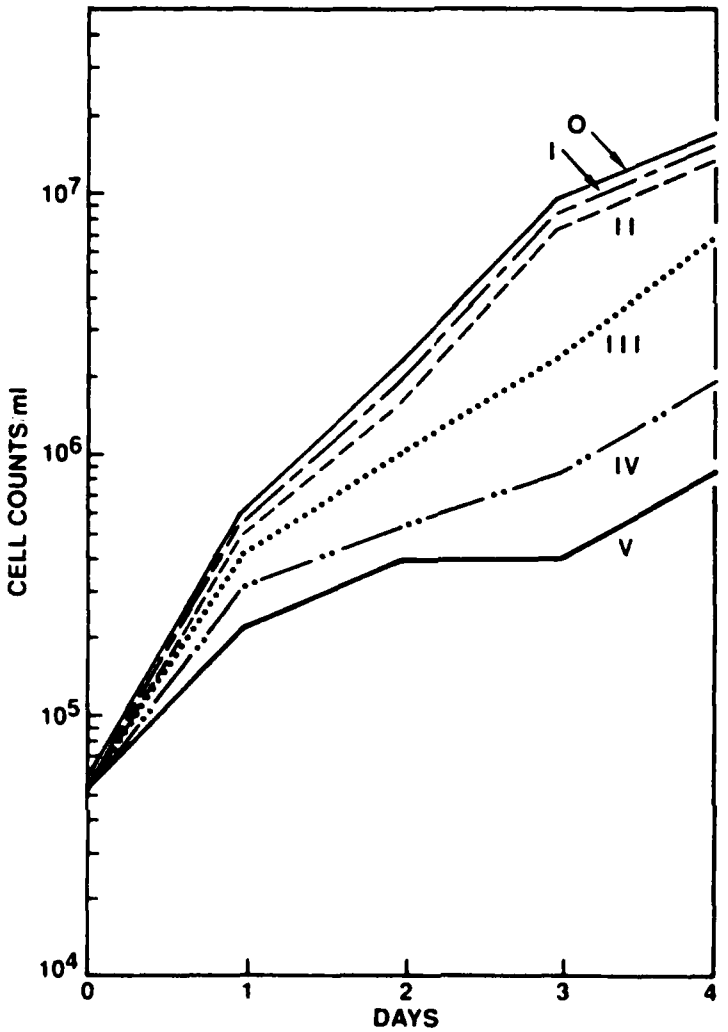

Figure 2. Effect of potassium dichromate ($K_2Cr_2O_7$) on the growth of *Scenedesmus subspicatus*. O = 0, I = 0.25, II = 0.35, III = 0.50, IV = 0.70, and V = 1.0 mg of $K_2Cr_2O_7$/l (Wong and Couture, 1986).

24 hours of incubation using *in vivo* fluorescence and DCMU-induced (the herbicide 3-(3,4-dichlorophenyl)-1, 1-dimethyl urea) fluorescence (Wong and Couture, 1986). The principle behind this technique is based on levels of photosynthetic energy produced by the growing population and its use. Some of the energy is lost as heat while some of the energy is lost in the form of chlorophyll *a* fluorescence at 682 nm. The loss of energy varies with the physiological state of cell population; minimal energy is lost for healthy cell populations and a significant amount is lost if the toxicant interferes with normal photosynthesis. The added DCMU will block the electron transport pathway in the light reactions of photosynthesis resulting in a maximum level of chlorophyll *a* fluorescence. The inhibition index, or the change in fluorescence obtained by subtracting *in vivo* fluorescence from DCMU-induced fluorescence, is directly related to increased toxic effect. Therefore, the inhibition index can be used as a bioindicator of the health of the algal population under study (Samuelsson *et al.*, 1978).

All of these measurements can be performed with available fluorometers. The results of this toxicity test are usually reported as an inhibition index following 24 hours of incubation and can be plotted as an EC_{50} (effective concentration of toxicant that decreases 50 percent of the fluorescence of the test algae) fitted line (figure 3). This technique may be useful in describing sublethal effects from toxicity of chemicals on algal populations. The advantage of using this technique is the short incubation time of the test (24 hours). This technique also shows promise because it retains the simplicity of *in vivo* fluorescence measurements and provides an estimate of photosynthesis which correlates well with ^{14}C uptake measurements (Rehnberg *et al.*, 1982; Samuelsson and Oquist, 1977).

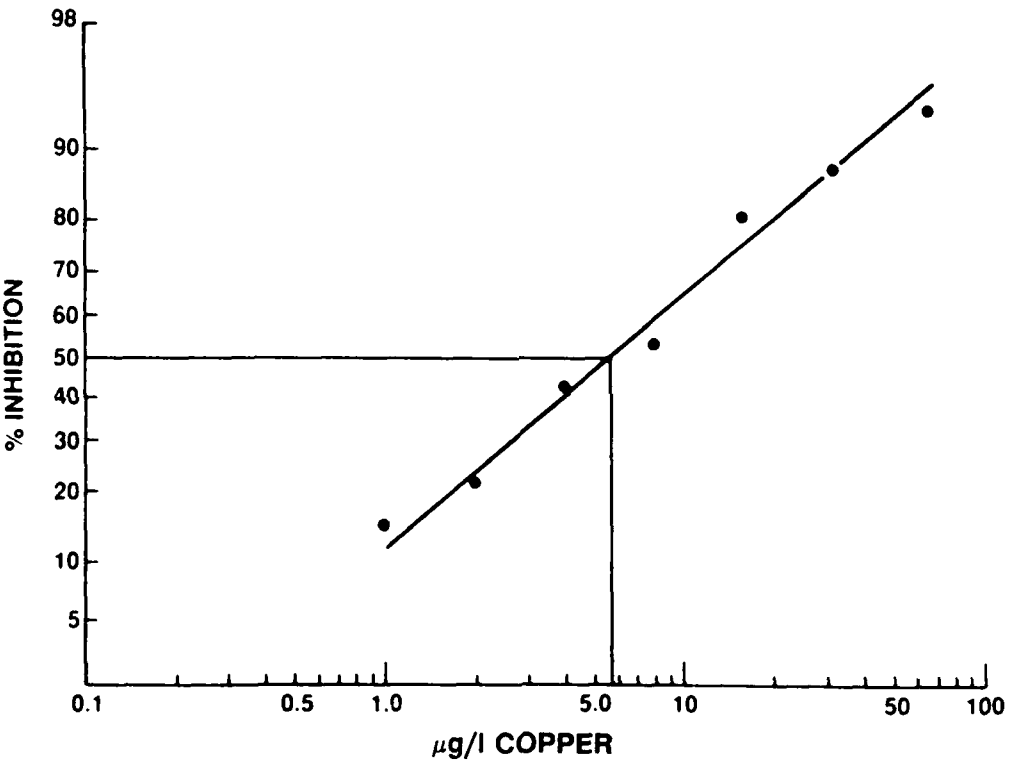

Figure 3. Estimation of 24-hour EC_{50} value by probit analysis and line of best fit. The data are calculated from fluorescence reported as an inhibition index. Both the percent inhibition and the concentration of copper are plotted on a logarithmic scale to obtain a best fit linear equation relating copper concentration to inhibition index. The EC_{50} is obtained by interpolating the concentration at an inhibition index of 50 percent (Wong and Couture, 1986).

Relatively few algal species are employed as assay organisms and the species which are under culture are chosen for convenience and not for sensitivity to pollutants. There needs to be an expanded effort on screening algal species (both dinoflagellates and diatoms) for determining which species may be more sensitive and applicable for use in bioassays. Indigenous species at an impacted site may or may not be the best possible choice of a bioassay organism if other species are more sensitive to the toxicants present (Madsen, 1984; Walsh and Merrill, 1984). Because of the complex and unknown

behavior of multiple contaminants simultaneously impacting the biota, the effects upon algae cannot be predicted solely from chemical composition because the observed effect in the biota is the result of the additive and synergistic behavior of the chemicals in relation to physical properties of the environment. Consequently, algae have been recommended as more sensitive indicators of complex toxins than more complex organisms and should be considered for evaluations of potential hazards (Walsh and Merrill, 1984).

THE USE OF CRUSTACEANS IN TOXICITY TESTING

Many species of crustaceans have been used to test the toxicological impacts of heavy metals, pesticides, and other contaminants. The parameters most often measured in crustacean bioassays are percent survival, growth rates of populations, number of adults reaching reproductive age, number of larvae released by females, and numbers of progeny surviving.

Toxicity tests using a variety of crustacean species have been conducted on a wide range of pollutants from heavy metals to organic pesticides. Studies have included the acute toxicity from Bis(tributyltin) oxide (TBTO) on the calanoid copepod *Acartia tonsa* (U'ren, 1983); the toxicity of cadmium to the life stages of the freshwater crustacean *Assellus aquaticus* (Green *et al.*, 1986); dieldrin effects on the development of two species of crab larvae (Epifanio, 1971); DDT effects on the amphipod *Gammarus pseudolimnaeous*; the effects of organochloride pesticides (Butler and Springer, 1963; Lowe, 1965; Eisler, 1969); the effects of pesticides on crab larval stages (Bookhout and Costlow, 1970; Buchanan *et al.*, 1970); and the toxic effects of sanitary landfill leachate, pure compounds, and complex effluents on the freshwater water flea *Daphnia magna* (Qureshi *et al.*, 1982; Neiheisel *et al.*, 1983; Plotkin and Ram, 1984). The estuarine epibenthic mysids *Mysidopsis bahia, M. bigelowi* (Nimmo *et al.*, 1977; S. Gentile *et al.*, 1982, J. Gentile *et al.*, 1983; Lussier *et al.*, 1985), and *Acanthomysis sculpta* (Davidson *et al.*, 1986) have been extensively studied in both acute and chronic bioassays because of their ecological importance as a food source for bottom-feeding fish (Markle and Grant, 1970; Odum and Heald, 1972). Their culture requirements have been worked out in the laboratory (Nimmo *et al.*, 1977).

The effects of cadmium on different life stages of the fresh water isopod, *Asellus aquaticus*, illustrates the importance of assessing the effects of toxicity on organisms over their life cycle (Green *et al.*, 1986). The sensitivity of *Asellus aquaticus* to cadmium exposure is dependent on the life stage of the organism (figure 4). The embryo and adult stages are more resistant than the larval and early juvenile states. Therefore, determining a "safe" exposure level for a given species requires an assessment which includes the life history aspects of the species. This is especially true for species such as *Asellus aquaticus* because seasonal hatching and maturation occur simultaneously for the whole population. A significant pollution event when the *Asellus aquaticus* population is in the early larval stages could eliminate this important species from the aquatic food chain (Green *et. al.*, 1986).

The study observing the effect of TBTO on the near-shore copepod, *Acartia tonsa*, is interesting for a variety of reasons. *Acartia* and all other copepods are some of the most abundant grazers in the food chain and can comprise as much as 70 percent of all zooplankton. An important food for larval fish, *Acartia tonsa* is recommended as a standard bioassay organism (APHA, 1980). The 96-hour LC_{50} (U'Ren, 1983) was

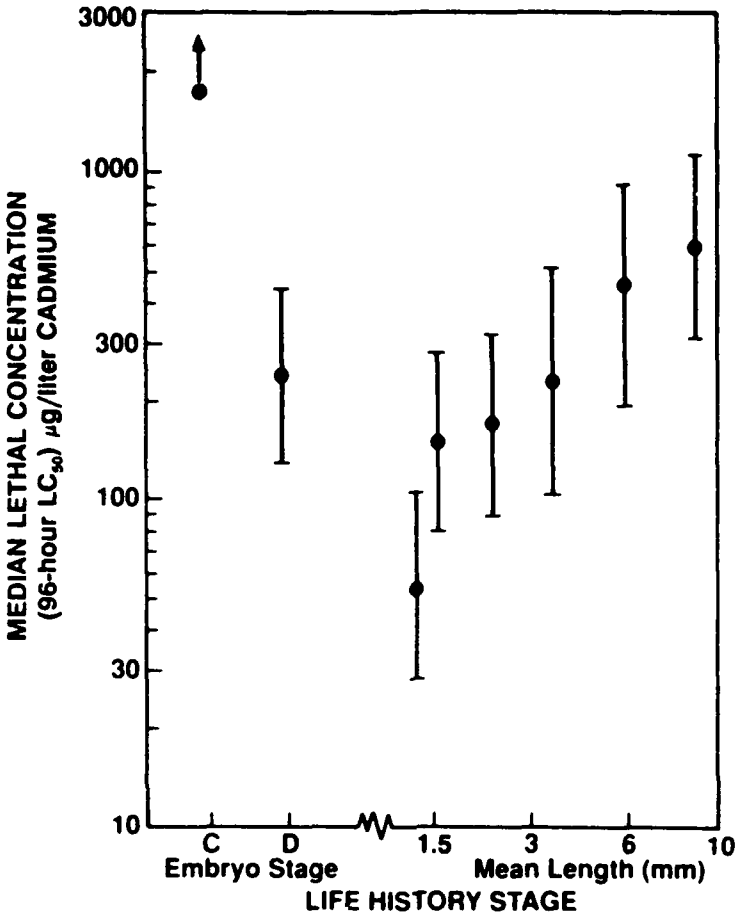

Figure 4. Median lethal concentrations (96-hour LC_{50}) for different life history stages of *Asellus aquaticus* exposed to cadmium (Green *et al.*, 1986).

1.0 µg/l TBTO while the 144-hour EC_{50} was 0.4 µg/l TBTO (figure 5). The 2.5-fold difference between acute and chronic levels obtained for the same species illustrates the necessity of obtaining data on sublethal effects. *Acartia tonsa* appears to be more sensitive to TBTO exposure than other crustaceans. The 96-hr LC_{50} for the harpacticoid copepod, *Nitocra spinipes*, is 2.0 µg/l (Linden *et al.*, 1979) while the 48-hr LC_{50} for the freshwater crustacean, *Daphnia magna*, is 3.0 µg/l (Polster and Halacka, 1971, cited in U'Ren, 1983). *A. tonsa* is more sensitive to TBTO than lobster larvae (5.0 µg/l; Laughlin and French, 1980), sheephead minnow, *Cyprinodon* (21 day LC_{50} is 0.96 µg/l; Ward *et al.*, 1981), and the rainbow trout *Salmo gairdneri* (24-hour EC_{50} is 30.8 µg/l; Chliamovitch and Kuhn, 1977). The advantages of using these minute crustaceans are their apparent hardiness in the laboratory, the simplicity of the assay system (visual counts of live, moribund, and dead), and the short duration of the assay (a few days).

The ultimate sink in the aquatic environment for heavy metals is the sediment. Several species of mysid crustaceans have been extensively used to monitor toxic compounds and heavy metals in bay and harbor sediments (S. Gentile *et al.*, 1982; J. Gentile *et al.*, 1983; Lussier *et al.*, 1985; Salazar and Salazar, 1985a, 1985b; Davidson

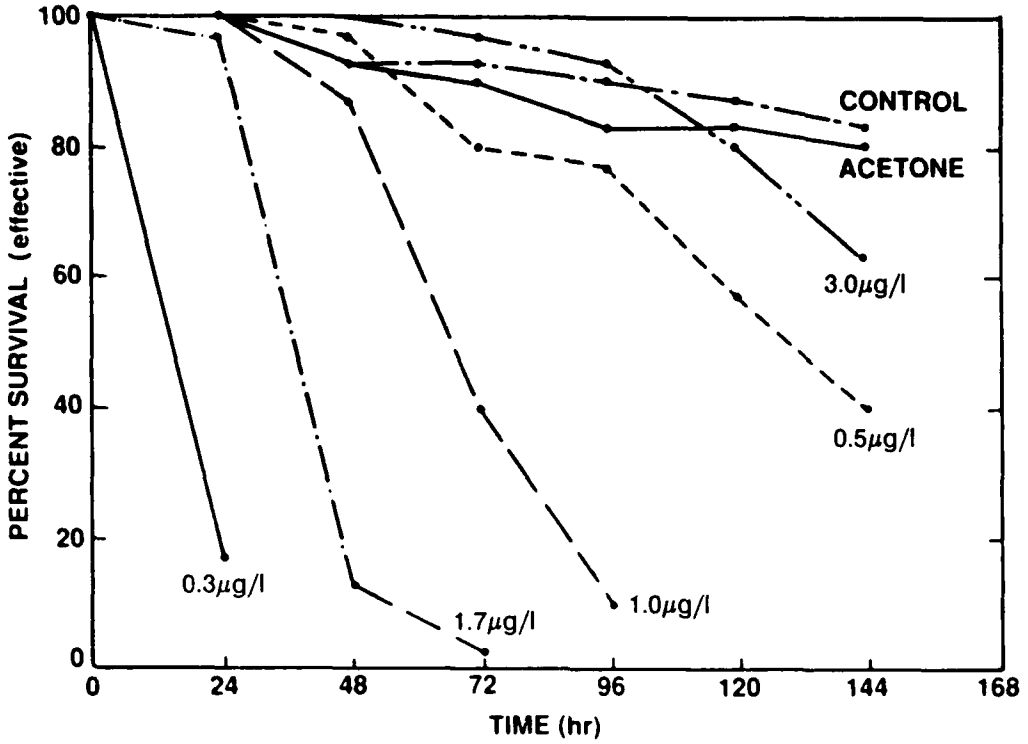

Figure 5. Effective survival of copepods (*Acartia tonsa*) exposed to five concentrations of TBTO (U'Ren, 1983).

et al., 1986). Other marine sediment assays use a short, acute static test to measure mortality and behavioral effects of phoxocephalid amphipods (Swartz *et al.*, 1985). In these tests, the emergence rate of the buried amphipod is dependent on the concentration of contaminants in the sediment. The relative toxicities of marine sediments can be assessed through these short, static bioassays.

Acute and chronic effects of other heavy metals on *Mysidopsis bahia* and *M. bigelowi* have also been documented (Lussier *et al.*, 1985; S. Gentile *et al.*, 1982). The 96-hour LC_{50} acute toxicity values range from 3.5 μg/l for mercury to 3130 μg/l for lead. Chronic toxicity values ranged from 1.2 μg/l for mercury to 893 μg/l for arsenic. Survival and number of young produced were effected by silver, arsenic, copper, and cadmium. Additionally, morphological aberrations were observed in both *M. bahia* and *M. bigelowi* exposed to cadmium at the onset of sexual maturity. The overall order of toxicity by ranking was: Hg>Cd>Cu>Cyanide>Ag>Zn>Ni>As>Cr>Pb (Lussier *et al.*, 1985).

The method of exposure and the conditions within the test tanks may influence the outcome of the bioassay. The influence of sediment on the survival of mysids exposed to organotins was investigated to determine the importance of sediment in reducing the bioavailability of organotin (Salazar and Salazar, 1985b). In a 10-day flow-through toxicity test (figure 6), mysids were exposed to tributyltin (TBT) levels of 0.4 to 0.8 μg/l in the presence and absence of sediment (figure 7). Both sediment and toxicant effected the survival of mysids. However, sediment enhanced mysid survival which implies that sediment is important in reducing the bioavailability of organotins to marine organisms.

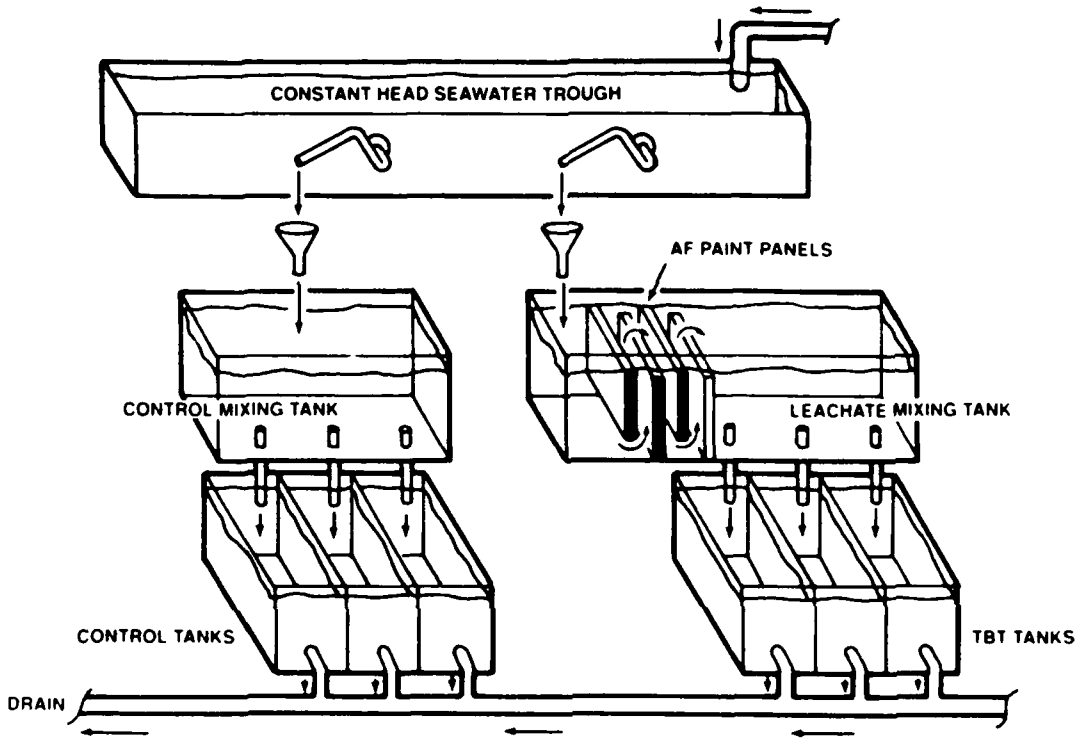

Figure 6. Flow-through seawater system used to deliver tributyltin (TBT) to test tanks. Seawater is forced to flow over a series of TBT-coated plexiglass panels at a rate of 6 l/min before distribution to test tanks (Salazar and Salazar, 1985b).

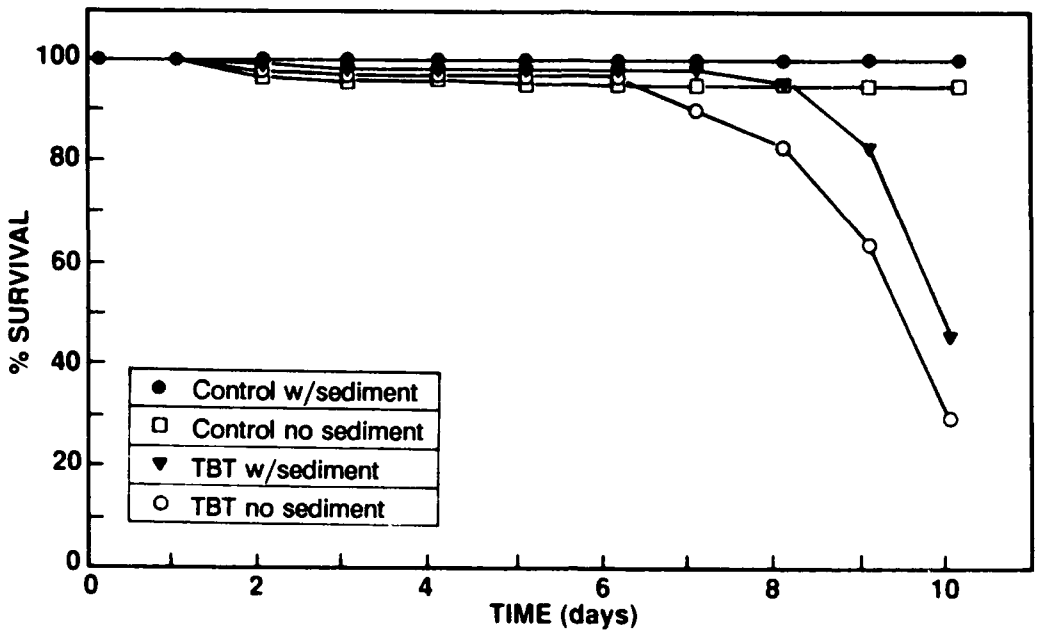

Figure 7. Sediment and toxicant both effect mysid survival. Sediment became a statistically significant factor in mysid survival by day 7 and mysids exposed to TBT exhibited higher survival in tanks with sediment than in tanks without sediment. After 10 days, control survival was 100% with sediment and 95% without sediment; TBT treatment survival was 46% with sediment and 30% without sediment (Salazar and Salazar, 1985b).

The differences obtained from short-term and long-term toxicity tests can be illustrated with data obtained from acute and chronic assays on the effect of TBT on the mysid *Acanthomysis sculpta* (Davidson *et al.*, 1986). The acute test, which was conducted over a 150-hour period (figure 8), obtained a 96-hour LC_{50} of 0.42 μg/l of TBT. The chronic test, which was conducted over a 60-day period (figure 9), resulted in a long-term chronic mortality value of 0.31 μg/l (table 1). The chronic test also revealed sublethal effects such as reduced growth and reproductive failure which were lower than the acute LC_{50} (table 1). The acute test was of short duration and employed static renewal or batch preparations of the test concentrations each 24-hour period. The long-term chronic test employed a flow-through system and provided a more realistic exposure to test concentrations. Both tests determined the significant effects by comparing the relative differences between test concentrations and controls.

Sublethal effects such as inhibited growth can be viewed as increased stress on an organism's struggle for survival. This leads to increased mortality. The lower limit for reproductive effects (table 1) may be due to the fact that females are already under reproductive stress and the additional effect of the toxicant is enough to cause significant differences at lower concentrations. The ultimate impact of reproductive failure and reduced growth from long-term exposure to low concentrations of pollutants may be much greater on the mysid population than mortality from short-term exposure to higher concentrations of pollutants. These factors must be taken into account to adequately define the margin of safety for pollutant exposure to populations of aquatic organisms at risk.

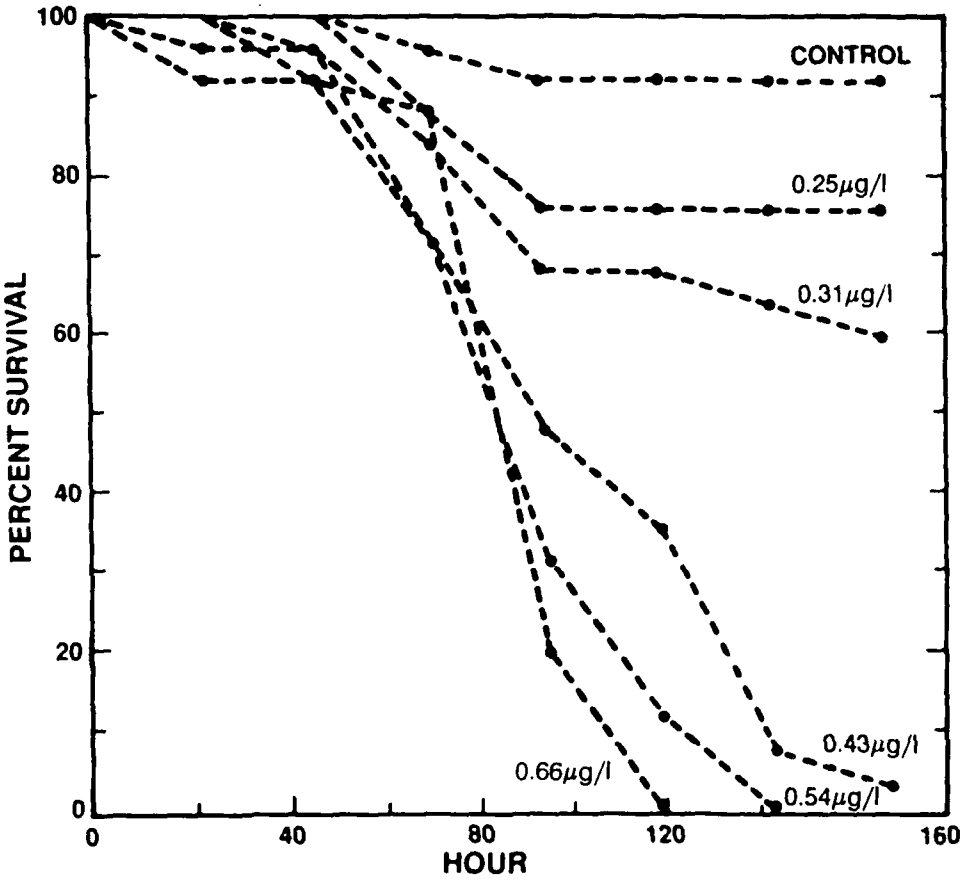

Figure 8. Percent survival through a 96-hour static renewal acute toxicity test of the mysid *Acanthomysis sculpta* (Davidson *et al.*, 1986).

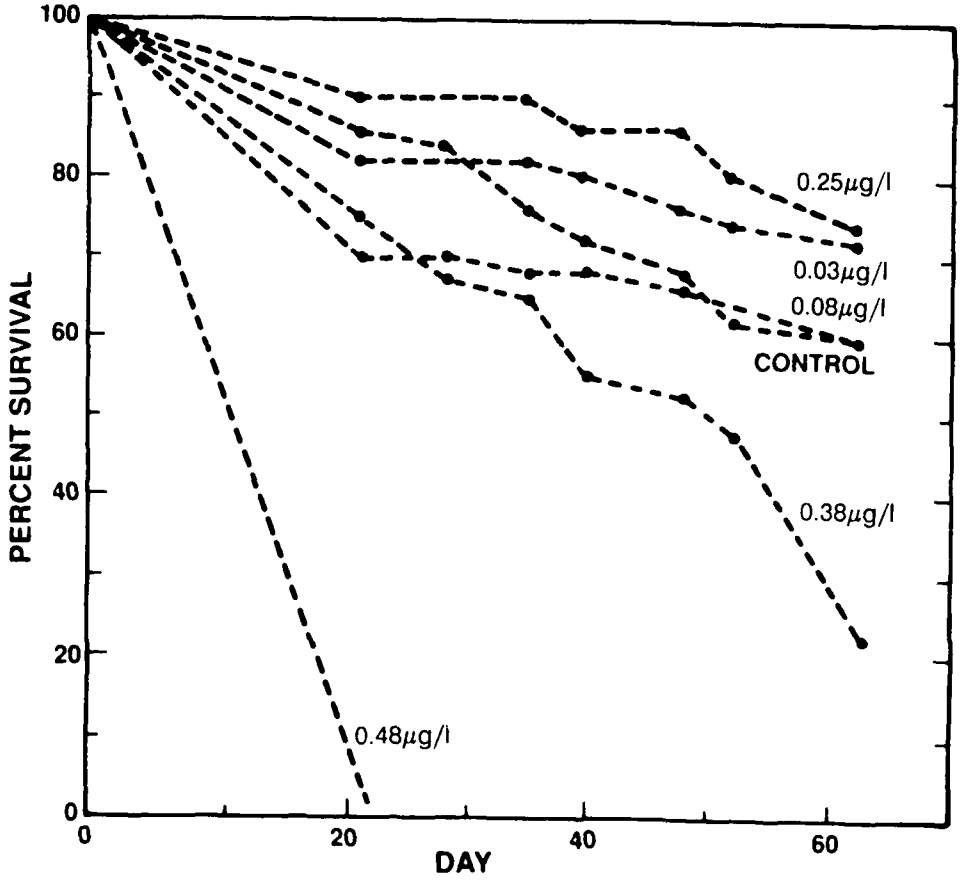

Figure 9. Percent survival through a long-term mysid mortality test (Davidson *et al.*, 1986).

Table 1. Summary of acute and chronic values in μg/l TBT for mysids exposed to concentrations of TBT. The final chronic value is determined as the average between the lower limit or no effects concentration (NOEC), which was the lowest concentration tested that caused a significant effect, and the upper limit, which was the highest concentration tested which did not cause an effect (Davidson *et al.*, 1986).

Test	Lower Limit (NOEC)	Upper Limit	Final Chronic Value
Long-Term Mortality	0.25	0.38	0.31
Growth			
Subadults	0.31	0.60	0.45
Adults (Females)	0.25	0.38	0.31
Reproduction	0.09	0.19	0.14
Acute Toxicity	96-hour LC-50 = 0.42 μg/l TBT		

THE USE OF MOLLUSCS IN TOXICITY TESTING

Molluscs comprise a large group of organisms which have life styles and habitat requirements (crawling, creeping, sessile, pelagic; riverine, estuarine, bay, open coast) that make them attractive for use in bio-monitoring schemes. The common bay or blue mussel, *Mytilus edulis*, and other shellfish have been the object of intensive research for decades because of their ability to concentrate heavy metals from seawater and, in a sense, act as sentry organisms which monitor water pollution.

Bivalve molluscs (the mussels *Mytilus edulis, M. californianus, M. galloprovincialis* and the oysters *Crassostrea* and *Ostrea*) are used in pollution assessments because they are cosmopolitan in distribution, sedentary in nature, and perhaps are better integrators of chemical pollution than are pelagic, mobile species. They have reasonably high tolerances to many pollutants with respect to crustaceans and fish and they can concentrate many pollutants from seawater by factors of 10^2 to 10^5. Lastly, they can be transplanted and maintained in bags and cages in areas where they are not normally found for environmental monitoring.

A program was set up in the United States to monitor the outer coastal areas called the "Mussel Watch Program" (Butler *et al.*, 1971). In the years of 1976 to 1978, 100 mussel tissue stations were sampled along the east and west coasts each year for heavy metals, radionuclides, fossil fuel hydrocarbons, and PCBs (Farrington *et al.*, 1987). Aspects related to stress of the accumulated chemicals in the shellfish were assessed ("The International Mussel Watch", 1980). Recommendations include using "scope for growth" measurements to assess stress in bivalve populations, the latency of lysosome enzymes in the cytotoxic response in mussels, the incidence of gonadal and hemopoietic neoplasms, and pathological damage to gill, kidney, and digestive gland tissue.

The Scope For Growth (SFG) index is a measure of the energy available to an organism for production of both somatic and reproductive maintenance (Warren and

Davis, 1967). The SFG value represents the instantaneous assessment of energy balance in an organism for that set of environmental conditions under which it is measured. The SFG measurements include (1) clearance rate or volume of water completely cleared of particles of a certain size in some unit of time; (2) respiration rate of single animals; (3) food absorption efficiency; and (4) ammonia excretion rates. Removal of particles and respiration rate are the easiest and quickest to measure.

There are limitations in the use of bivalves for accumulation studies and extrapolation to the health of the entire ecosystem. A major problem is to extrapolate body burdens of chemicals in bivalves to effects on human health. The use of bivalves as sentinel organisms is of limited use because they do not appreciably bioconcentrate water-soluble chlorinated pesticides such as hexachlorocyclohexane (HCH) and toxaphene. There is a variety of factors which influence the rate of uptake and concentration of certain chemicals in bivalves such as reproductive status, nutrition, temperature, salinity, and particulate matter in the water column (Farrington *et al.*, 1987; Amiard-Triquet *et al.*, 1986).

Numerous studies have been conducted to evaluate the effect of TBT on populations of the bay mussel, *Mytilus edulis*. In a study to define the long-term toxicity and bioaccumulation of TBT (Valkirs *et al.*, 1985) populations of mussels were exposed to test concentrations of TBT that ranged from 0.04 to 1.89 µg/l TBT (figure 10A).

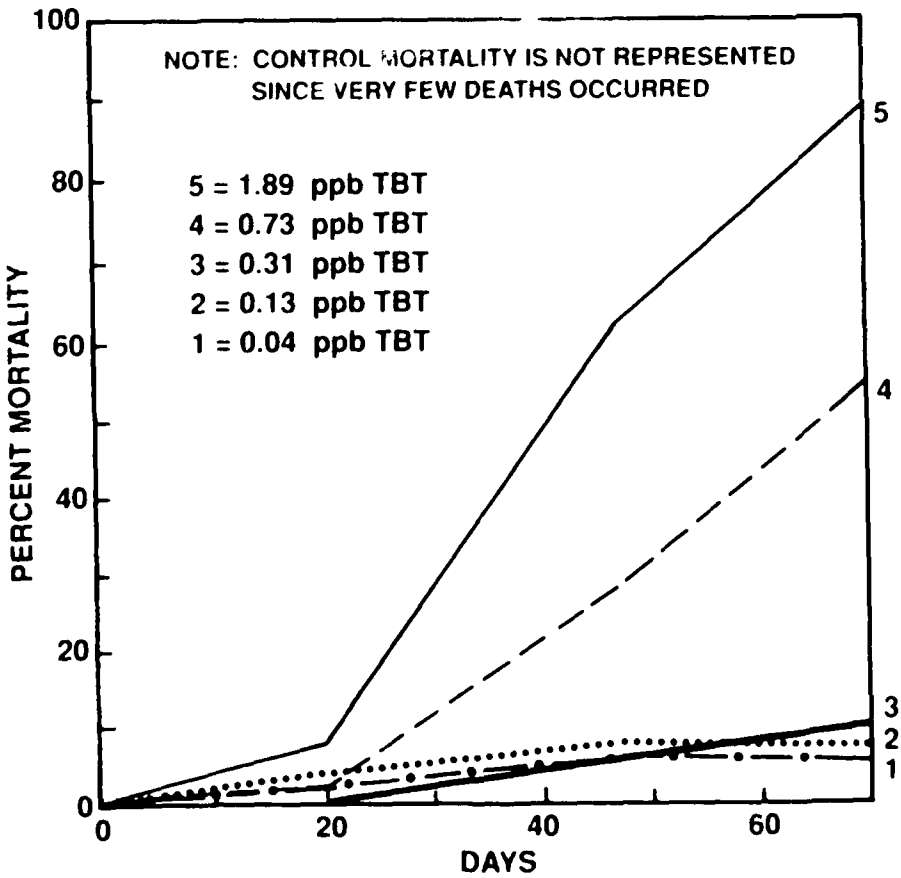

Figure 10A. Mussel cumulative mortality recorded during long-term toxicity test (Valkirs *et al.*, 1985).

Significant differences in growth and mortality did not occur until after 20 days of exposure. After day 20 the mortality rate of mussels exposed to the highest TBT concentrations increased dramatically (figure 10A). The chronic LC_{50} value of 0.97 µg/l TBT was computed by probit analysis of the data obtained from the 66-day exposure (figure 10B). The probit curve shows the probability of death for a given percentage of the test population as a function of exposure. The sharp increase in the probability of lethal exposure at 0.50 µg/l TBT reflects the dose-response threshold determined from this set of data (figure 10B). The results obtained from this study shows it is important to conduct long-term bioassay tests to obtain realistic assessments of environmental toxicity (Valkirs *et al.*, 1985).

Toxicity tests show mussel larvae are extremely sensitive to TBT exposure. In a laboratory experiment 50 percent of the *Mytilus edulis* larvae exposed to 0.10 µg/l TBT died by day 15 of the experiment (Beaumont and Budd, 1984). However, the increased stress as a result of conducting laboratory toxicity tests raises the question as to whether laboratory results are applicable to actual field exposures.

One approach of narrowing the gap between laboratory and field studies is to use portable microcosms which can be deployed at specific locations to test the responses of aquatic organisms to toxicants under near-natural conditions (Henderson, 1985; Salazar and Salazar, 1987). Flow-through microcosms located on San Diego Bay, CA were used to evaluate the effect of TBT concentrations ranging from 0.04 to 0.20 µg/l on mortality and shell growth in juvenile *Mytilus edulis* over a 60-day period. Shell growth and mortality were not effected at any of the test concentrations. The results of this study suggest the effects of TBT on juvenile mussels may have been overestimated from laboratory results (Salazar and Salazar, 1987).

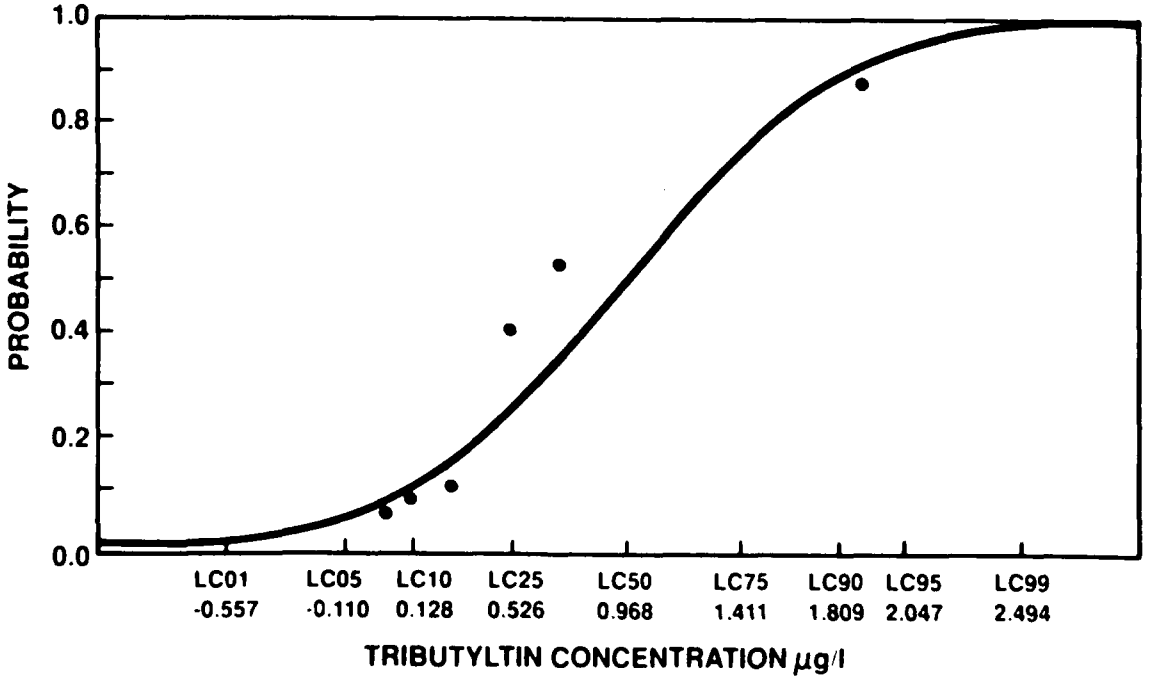

Figure 10B. Mussel mortality probit analysis (Valkirs *et al.*, 1985).

Perhaps by virtue of their habitat distributions, other molluscan species should be investigated as new tools for assessing impact to an aquatic site. The middle-intertidal marine gastropod, *Crepidula fornicata*, was investigated for the effects of silver on reproductive behavior of both the parental stock and F_1 generations (Nelson *et al.*, 1983). Advantages in using this animal for long-term, sublethal toxicity studies include local abundance, size, sedentary behavior, fecundity, and ease of culture in the laboratory (Calabrese and Rhodes, 1974). Because of the gastropods gregariousness and distribution in the rocky intertidal zone along both the Atlantic and Pacific coasts (different species), it may prove to be a useful biosensor for accumulation of heavy metals.

Fertilization success, larval life stages, and larval settling are all critical parameters important to a population's continued recruitment and stability. These aspects have been intensively studied in haliotid molluscs (abalone) over the past decade. It seems feasible to monitor these parameters as a function of the toxic concentration, either in the water or in the substrate, or in both. This type of assay requires minimal assets since the observations entail counting the number of larvae at each stage and comparing against the concentration of experimental chemicals (Martin *et al.*, 1986). It may also be prudent to continue chronic, long-term studies in the fishery to observe effects of the toxins on shell growth, weight gain, and scope for growth measurements in older abalone.

OTHER SPECIES TOXICITY TESTS: USE OF SEA URCHINS, MARINE WORMS, FISH, AND BIRDS IN TOXICITY TESTING

This section provides a quick overview of species for which toxicity testing is available and which might be required to assess impact at specific Navy hazardous waste sites. These tests include sea urchins, worms, fish, and bioassays using avian populations.

Numerous experimental observations have been documented describing the effects of various agents on the developmental stages in many species of sea urchins (Kobayashi, 1984). Sea urchins constitute an ideal organism for toxicity testing because they are found along most coastlines and the biology of spawning, fertilization, and larval development has been defined for many sea urchin species. Also, experimentation with adults, embryos, eggs, and sperm can be done routinely in the laboratory. Many of these bioassays examine the abnormal effects or delays in the routine development of eggs, sperm, and embryos for various species. Some of the advantages for using sea urchins in toxicity testing are the simplicity of the test, the short period of time needed to conduct the test (a few days), and the high sensitivity of the test. At least 25 species of sea urchins from around the world have been used in pollution bioassays. In the United States, the species commonly used are *Strongylocentrotus drobachiensis, S. purpuratus*, and *Arabacia punctulata*.

A variety of test methodologies has been worked out which examines abnormalities in the earliest phase of fertilization success in sea urchins. There are gamete toxicity tests (Hagstrom and Lonning, 1973) which examine the influence of a substance on the viability of eggs and sperm, a sperm toxicity test which quickly assesses whether eggs added to a sperm-toxicant solution are fertilized at normal percentages (Dinnel *et al.*, 1982), and larval tests whereby the success of both fertilization and subsequent development to the gastrula and pluteus stages in the test solution is measured (Bougis *et al.*, 1979).

Heavy metals have been observed to inhibit fertilization success and arrest development in early developmental stages in sea urchins (Kobayashi, 1984). At high metal concentrations, fertilization of the gametes does not occur and eggs may even disintegrate. Retarded development of some stages in sea urchins has also been observed at low metal concentrations. Mixtures of heavy metals are more toxic to the developmental stages of sea urchins than single metals. Threshold concentrations of metals (Hg, Cu, Zn, Ni, Pb, Cr, and Mn) which arrest fertilization and development are at the part per million (ppm) level (Kobayashi, 1977; Kobayashi and Fujinaga, 1976).

Polychaete annelids, or marine worms, have only recently been used in bioassays. There are 22 species of annelids which have been used but *Neanthes arenaceodentata* and *Capitella capitata* are employed most frequently (Reish *et al.*, 1983). The most common type of bioassay is to place one worm into a dish containing the test solution either for 96 hours (acute bioassay) or for as long as 28 days (chronic bioassay). The 96-hour LC_{50} (lethal concentration which kills 50 percent of the test population in 96 hours) is commonly used because it is easy to conduct, results are rapidly obtained, and comparisons are feasible since many other bioassays are also conducted for 96 hours. Flow-through bioassays are also employed in the laboratory for greater field realism, however, flow through systems generally require more facilities, more seawater, and a complex delivery system (Reish *et al.*, 1983).

The 96-hour LC_{50} data indicate the effects of heavy metals on polychaetes occur at the ppm level. Mercury and copper are the most toxic while zinc, lead, cadmium, and nickel are less toxic to polychaetes (Reish *et al.*, 1983). Effects on reproduction have been observed at high levels of metals. At intermediate levels of metals, the number of eggs laid by the worms was reduced. Even relatively low concentrations of copper have induced abnormal larval development (Reish and Carr, 1978).

The advantages of using marine worms for bioassays are numerous. The worms are abundant along all coasts and bays and easy to collect. They are soft-bodied animals which allows their tissue to come into direct contact with contaminated sediments. Space requirements are minimal to house these animals and they are easy to breed in the laboratory. Their short life cycles make them an attractive candidate for studying the effects of various toxicants on reproduction (Reish, 1980).

Historically, fish species have been used to identify acutely toxic discharges (Carter, 1984). Widespread use of marine fish species in bioassays has lagged behind the use of freshwater fish and other marine invertebrates because of the difficulty of obtaining suitable marine species for testing throughout the year. The basic maintenance of these species requires a good supply of seawater which is free of harmful pathogens and filtered to remove suspended sediment. Their life cycles often extend over many months or years, so studies concerned with growth rates or reproductive capacity can last for long periods of time. This is costly from a manpower and laboratory resource point-of-view. Nevertheless, fish bioassays are still necessary to determine if a substance is harmful to fish stocks.

Acute and chronic bioassays are the primary tests conducted on fish species. Acute tests usually use a 96-hour LC_{50} while chronic tests may last several weeks. Acute tests use the number of fish deaths as an end point. Long-term chronic tests evaluate the toxicant's effect on behavior, growth, and reproduction.

Some of the most common species of fish used in bioassays are the sheephead minnow *(Cyprinodon variegatus)*, the killifish *(Fundulus heteroclitus)*, the coho salmon *(Oncorhynchus kisutch)*, the three spine stickelback *(Gasterosteus aculeatus)*, the plaice

(Pleuronectes platessa), and the flounder *(Platichthys flesus)*. The sheephead minnow and the killifish are commonly used for both acute and chronic studies. The acute lethal tests are usually run for 96 hours while the chronic tests may run from 4 to 28 days and longer. The end point used for both juvenile and adult Coho salmon is mortality (Chapman and Stevens, 1978; Sugatt, 1980); in *Pleuronectes*, fertilization success (Nagell *et al.*, 1974; Lonning, 1977; Lonning and Hagstrom, 1976); and for adult flounder, observed hyperactivity (Bengtsson and Larsson, 1981), altered carbohydrate metabolism (Larsson and Haux, 1982), and fertilization and development (Lonning and Hagstrom, 1976).

Many Navy hazardous waste sites are located in sensitive marshland and upland areas. Bird species like starlings *(Sturnus vulgaris)*, hawks *(Buteo jamaicensis)*, and herons *(Ardea herodias)* are ideal for assessing impacts to wildlife (Kendall, 1988). NOSC is currently sponsoring a toxicology demonstration project using enhanced avian bioassays at the Naval Air Station Whidbey Island, WA (Johnston *et al.*, 1988). This bioassay uses starlings as bioindicators of toxicological effects by establishing a quadrat of nest boxes to create a gradient of exposure from the hazardous waste site. Toxicological effects on survival, behavior, bioaccumulation, and reproductive success can be determined and evaluated for significant impacts by comparing results obtained along the gradient. Toxicants of concern can be identified by analysis of feeding behavior and bioaccumulation data (Brewer *et al.*, 1988; Robinson *et al.*, 1988). In addition, biochemical levels in the serum of raptors, tissue burdens of contaminants in rodents, and toxicological impacts to a local colony of great blue herons will also be evaluated to determine whether contaminants from the waste sites are moving into the food chain and significantly effecting wildlife. Successful demonstration of this approach will provide methods for directly measuring environmental health at hazardous waste sites and aid in the development of remediation and risk management plans.

METHODS FOR MEASURING POLLUTION IMPACTS AT THE POPULATION AND COMMUNITY LEVEL

Pollution impact on an ecosystem will cause a shift in the resources available to organisms. This results in a change in the ability of the environment to provide the specific ecological requirements needed for a species to develop, mature, and produce offspring. Changes in the numbers of species, abundances, and shifts in the dominance of some species over others have been attributed to impacts from pollution (Gray, 1981; Pearson, 1975; Washington, 1984; Warwick, 1986; Henderson, 1985; Tagatz *et al.*, 1986). Changes in community structure and composition may provide powerful information on how contaminants impact aquatic ecosystems. It is difficult, however, to resolve the effects of pollutants from the natural spatial and temporal variability of the ecosystem (Warwick, 1986).

The usual approach in detecting pollution effects at the community level is to use some measure of community composition such as diversity or similarity indices (Washington, 1984), or to use data for indicator species whose presence, absence, or abundance relates environmental quality to laboratory derived toxicity levels for that species (Washington, 1984; EPA, 1976). Diversity or similarity indices are used to provide a measure of how individual organisms (abundance) are divided between numbers of species (richness) (Washington, 1984). Differences in diversity indices between impacted and nonimpacted sites indicate pollution influence on the species composition of stressed communities. Changes in diversity indices are useful for flagging

sites for more detailed investigations, however, this alone does not provide any information about how pollution impacts the important biological processes of the community (Hurlbert, 1971).

Reliance on presence or absence of indicator species works well only for specific toxicants whose fate and effect is known. A wide range of tolerances exists among species and the use of a particular species as an indicator should adequately represent the toxicity effect. For example, mercuric chloride levels of 50 μg/l may not significantly effect the polychaete worm population *(Ophrytocha diadena*, LC_{50} 71 μg/l; Reish and Carr, 1978) but it may have eliminated entire populations of colonial hydroids *(Campanularia flexuosa*, LC_{50} 1.6 μg/l; Stebbing, 1976), copepods *(Pseudocalanus minutus*, LC_{50} 5.0 μg/l; Sonntag and Greve, 1977) and mysids *(Mysidopsis bahia*, LC_{50} 1.1 μg/l; J. Gentile *et al.*, 1982). Furthermore, populations of indicator species may be highly influenced by natural variations in recruitment and predation.

A better approach is to use groups of species, or taxocene, to monitor for effects (Hurlbert, 1971). Groups of species used for monitoring could be chosen to reflect a variety of life history strategies (e.g., primary producers, filter feeders, deposit feeders, carnivores, detritivores, etc.). Taxonomic phyla could provide information on the effects of contaminants over a wide range of potential targets. The specific ecosystem and contamination source under study will determine which species to select.

Microcosms and mesocosms provide contained ecosystems which can be manipulated and replicated to test fate and effects of contaminants and assess changes in community composition due to pollution impact (Henderson, 1985; Donaghay, 1984; Levin, 1986; Salazar *et al.*, 1987). The tank effect, or the impact of keeping organisms in an artificial system, must be less than the pollution effect being measured within the microcosm/mesocosm to be able to discern the community response to pollution. Additionally, researchers must be able to detect the ecosystem response against the background of natural variability (Donaghay, 1984) by using appropriate field controls.

The systematic use of microcosms/mesocosms has become an important tool in evaluating ecosystem response to pollutants. The Marine Ecosystems Research Laboratory (MERL) is a mesocosm facility at the University of Rhode Island's Graduate School of Oceanography. The laboratory was established in 1976 with funding from EPA. The goals of MERL are to carry out coordinated, focused, whole-ecosystem experiments on the fate and effect of substances of interest. Long-term experiments have been conducted to observe ecosystems under the influence of hydrocarbons, enhanced nutrients, sewage sludge, and polluted sediments. Their experimental systems can be operated for relatively long periods of time over a seasonal or annual period (Marine Ecosystems Research Laboratory, 1987). Studies conducted at MERL show good correlations between variables in the mesocosm tanks and Narragansett Bay, RI, especially with nutrient cycling and eutrophication.

A portable microcosm system has been developed and tested by NOSC in Pearl Harbor, HI, and San Diego Bay, CA. This innovative approach allows manipulative experiments which measure pollution effects in a controlled system at specific sites. In an experiment conducted at Pearl Harbor, a microcosm consisting of 155 L flow-through tanks was used to measure the differences in recruitment of marine communities subjected to five concentrations of TBT (Henderson, 1985). The number of species per treatment and the diversity index were recorded during both an exposure and recovery phase. During the exposure phase, the number of species per treatment decreased with increasing concentrations of TBT (figure 11A). The diversity index also decreased with increasing concentration of TBT but there was more variation in the data (figure 11B).

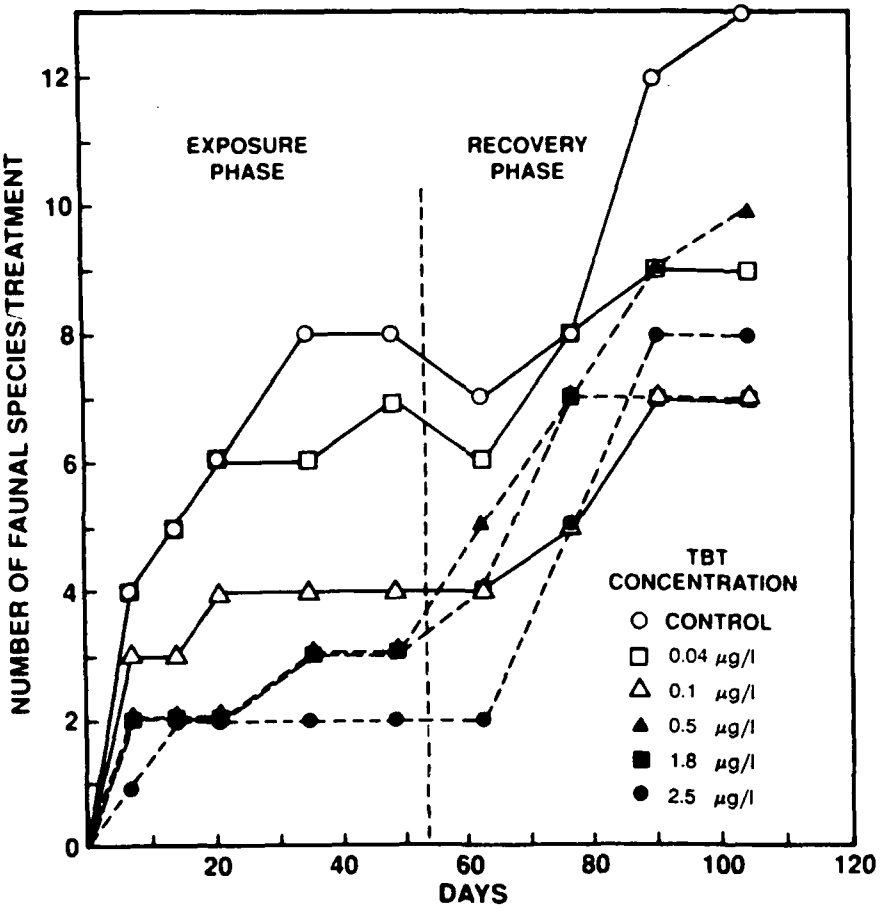

Figure 11A. Total number of faunal species on settlement panels plotted versus time for bioassay control and leachate treatments (Henderson, 1985).

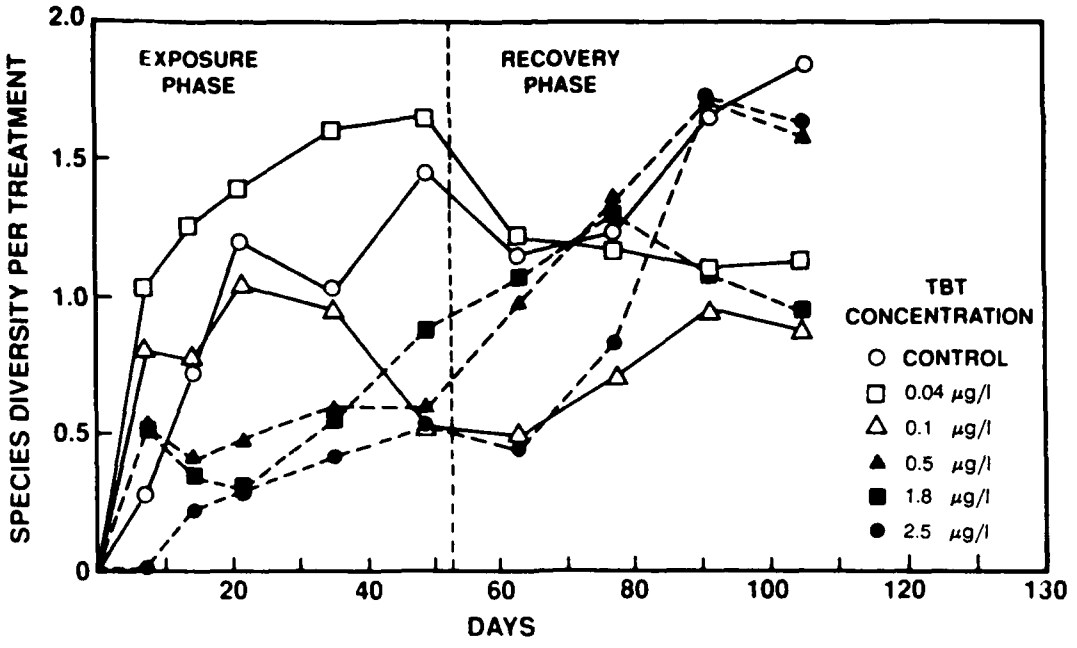

Figure 11B. Species diversity (modified Shannon-Weiner index) of fauna on settlement panels plotted versus time for bioassay control and leachate treatments (Henderson, 1985).

During the recovery phase, both the number of species per treatment and diversity index increased (Henderson, 1985). These data show the community responded to the stressed environment created by the presence of TBT, by the reduction of species, and by the selection of individuals more tolerant to the toxic conditions. When the toxicant was removed for the recovery phase, the increase in both numbers of species and diversity index shows the community was responding to the less stressful environmental conditions.

It is important to test the effects of toxicants in the field, especially when assessing the effects that may be occurring on the community level. In a study by Tagatz *et al.* (1986), the effect of dibutyl phythalate (DBP) on the recruitment of benthic infauna was investigated under field and laboratory conditions. Tagatz and his co-workers laced sediment with three concentrations of DBP dissolved in acetone. The laced sediment and control sediment containing only acetone were separated into containers and placed into flow-through aquariums at locations in Santa Rosa Sound, FL. Although the researchers found differences in the results obtained, it was difficult to quantify community differences and determine the ecological significance.

The task of relating the ecological significances of measured toxicity data depends on interpreting the ecological consequences of the insult. Toxicity effects are usually detected at concentrations much higher than those found in the natural environment. One could conclude there will be no effect to the natural population or assume the real effects are synergistic, more subtle, and harder to detect (Epifanio, 1984). More work must be accomplished before one can accurately relate mesocosm results to natural systems. Additionally, tools and methods must be developed to better understand and interpret ecological data.

One approach for evaluating samples of macro-invertebrates collected from benthic communities on an ecological basis was proposed by Warwick (1986). He proposed constructing k-dominance curves by plotting the cumulative percent of species biomass and species numbers as a function of species rank, or the arrangement of most abundant to least abundant of the species collected in the sample (figure 12). This model assumes that under unpolluted conditions the amount of biomass is dominated by one or only a few species consisting of large, competitively-dominant individuals in equilibrium with the available resources. The numerically dominant species are usually smaller organisms which are out of equilibrium with their resources and subject to random variations. These conditions will result in a biomass distribution strongly dominant (ie., will lie above the numbers distribution) because the numbers of individuals tend to be evenly distributed between the species as in figure 12A. Under moderately polluted conditions, some of the larger, competitively dominant individuals will be eliminated causing a change in the resource use in the community. This results in numerical and biomass distributions that are on top of each other or crossing (figure 12B). Under conditions of severe pollution, the community will be increasingly dominated by a few species that have greatly increased their numbers so that the numbers distribution is much higher than the biomass distribution (figure 12C).

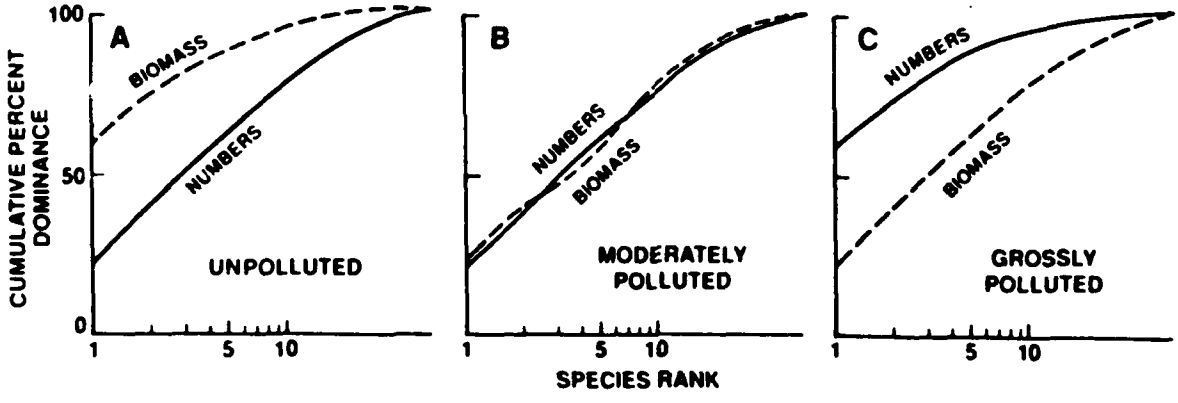

Figure 12. Hypothetical *k*-dominance curves for species biomass and numbers, showing unpolluted, moderately polluted, and grossly polluted conditions (Warwick, 1986).

The advantage of this method is field collections can be evaluated for evidence of pollution effects without the need for vast quantities of historical data. The disadvantage is the method needs adequate sampling in order to assure the results will not be biased by sampling error.

Warwick's method was applied to a set of data collected by Pearson (1975) over a 10-year period (1963 to 1973) at a Scottish Loch impacted by effluent from a pulp mill. Figure 13A shows the diversity index calculated from the data and indicates the start, increase, and decrease of effluent into the loch. The k-diversity curves compare to the change in diversity index. In the years 1963 through 1967, a nonpolluted condition was detected (figures 13B through F). During 1968 through 1970, a moderately polluted condition was detected (figures 13G through I), while 1971 and 1972 showed the characteristics of a severely polluted condition (figures 13J through K). The k-dominance

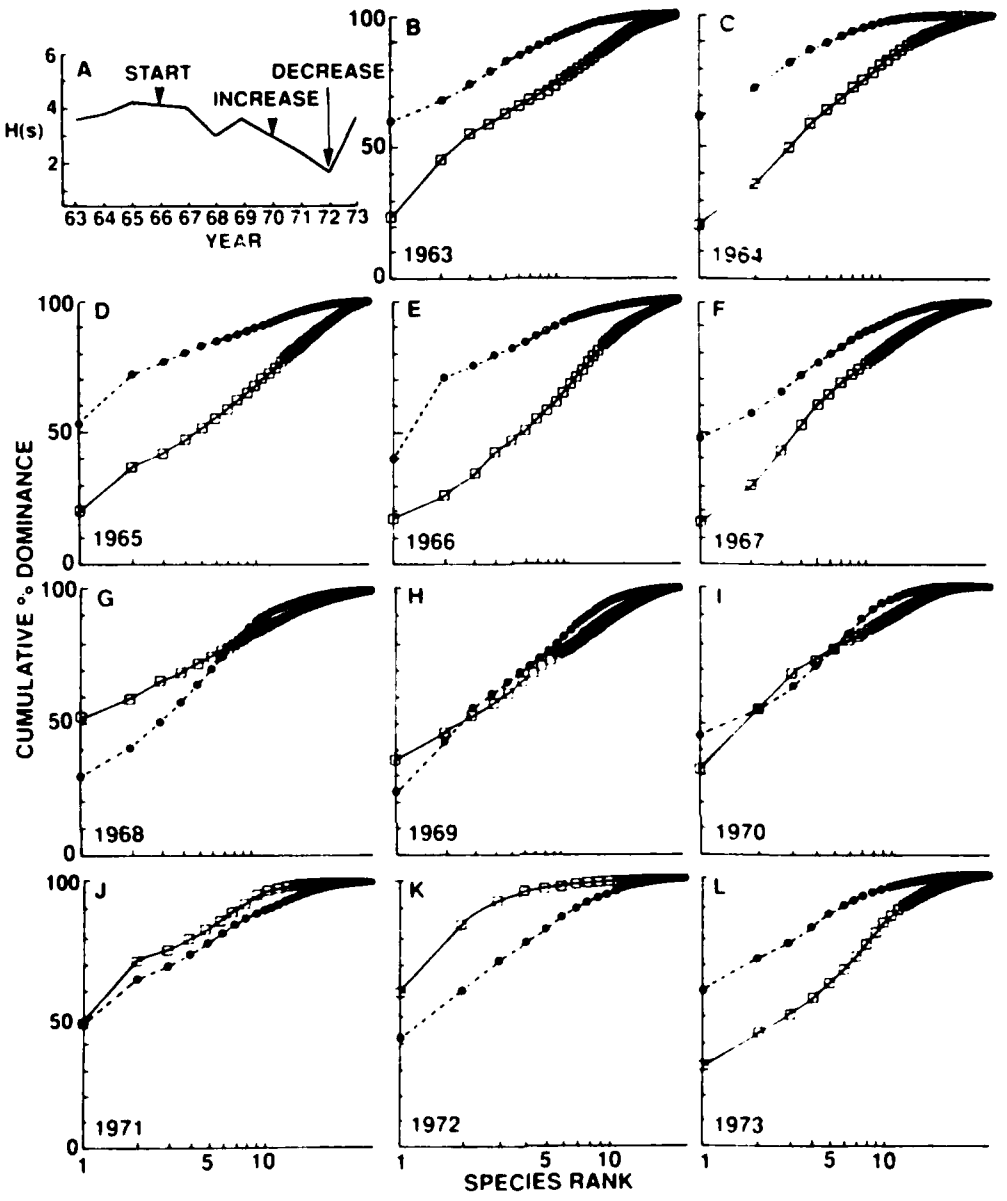

Figure 13. (A) Species diversity in Loch Linnhe, 1963-1973, showing timing of pollution events. (B–L), combined *k*-dominance curves for biomass (crosses and dashed lines) and numbers (squares and continuous lines) for the years 1963 to 1973 (Warwick, 1986).

curve obtained for 1973 (figure 13L) showed the unpolluted conditions had returned to the loch, correlating with the increase in diversity index shown in figure 13A. This method shows promise but must be applied to more field situations before its validity can be confirmed.

Another method which could be used to assess toxicity at the community level on a biological basis compares Whittaker dominance-diversity curves (Whittaker, 1965) generated from data from environments subjected to different levels of pollution. Dominance-diversity curves are generated by plotting an important biological measure,

such as net annual productivity or standing stock of biomass, versus the sequence of most abundant (in terms of the biological parameter) to least abundant species (figure 14). The shape of the curve relates to the partitioning of the environmental resources

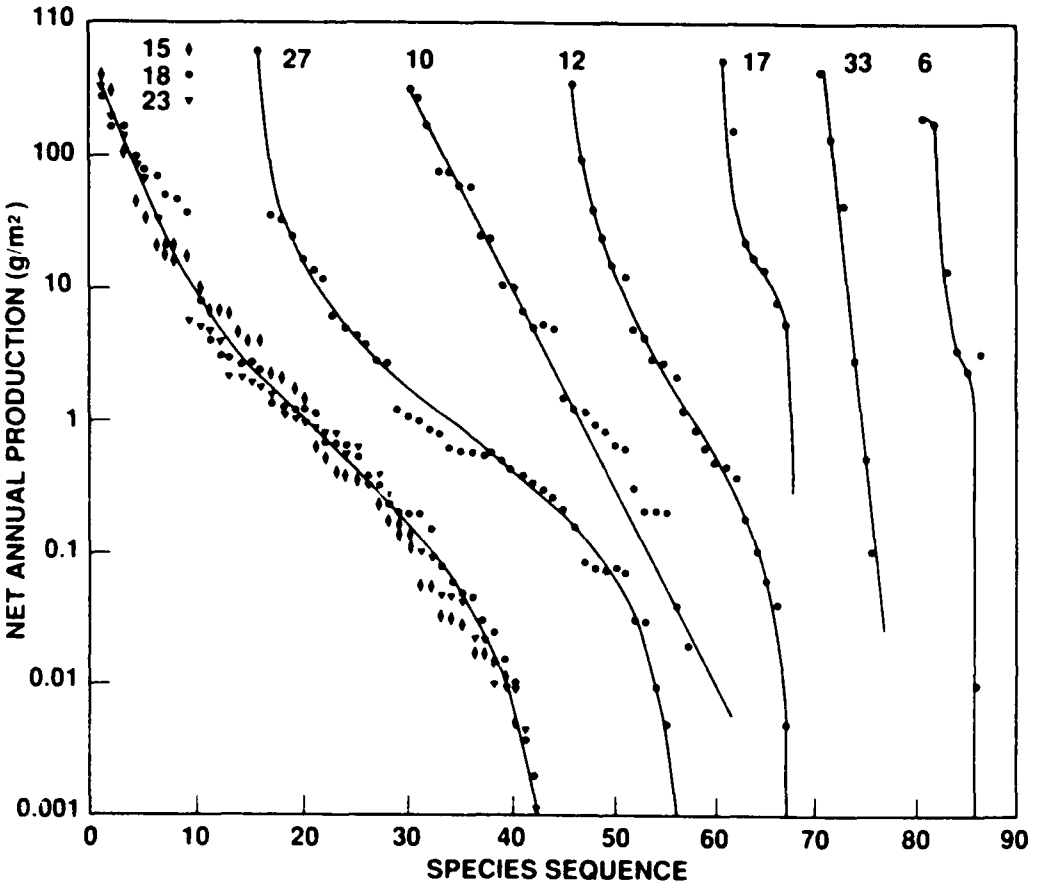

Figure 14. Dominance-diversity curves for vascular plant communities in the Great Smokey Mountains. Paints represent species plotted by net annual above-ground production (ordinate) against species sequence from most abundant to least abundant (abscissa). Sample numbers are listed at the top of each curve (Whittaker, 1965).

used by each species. Changes in the dominance-diversity curves can be used to interpret changes in community composition, such as the change in community composition occurring during colonization of experimental plates shown in figure 15 (Johnston, 1986). Dominance-diversity curves have also been used to evaluate differences in the composition of marine fouling communities along a pollution gradient in San Diego Bay, CA (Johnston, 1989).

The construction of life tables and survivorship curves may also be useful for interpreting population dynamics and the impact on community composition. Life tables can be constructed from mortality and survivorship data obtained from studies of life cycles of organisms (Deevey, 1947). Survivorship curves could be generated for a species subjected to both polluted and unpolluted conditions. The differences in the curves generated could provide a very useful analysis of the pollutant's effect on population

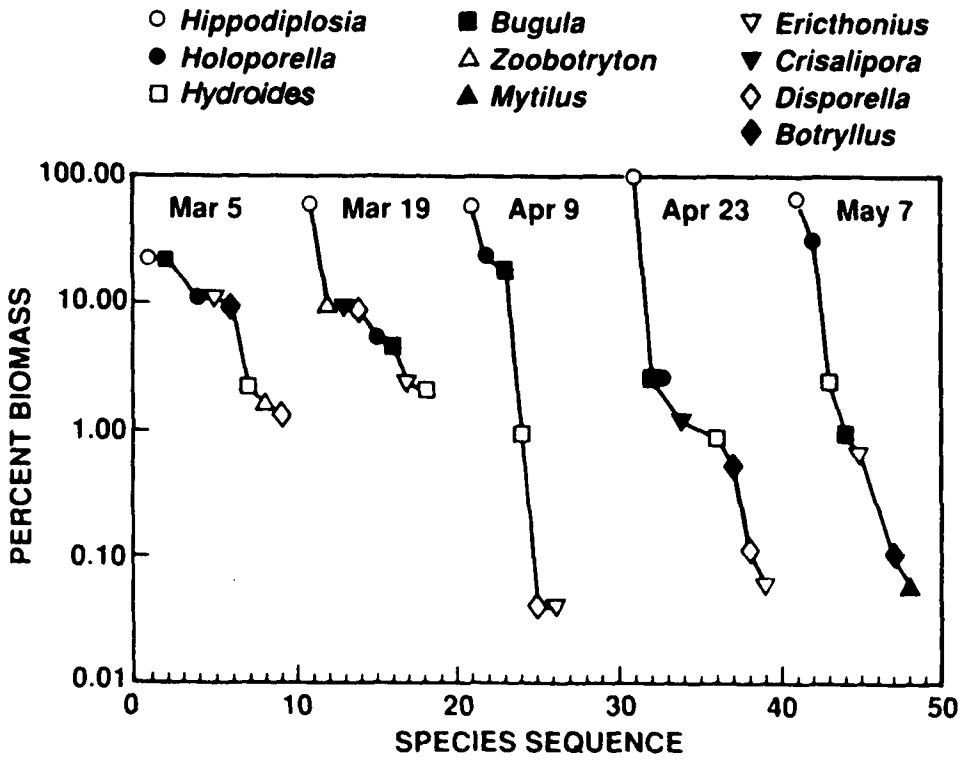

Figure 15. Average biomass from replicate plates collected from Mission Bay, CA. Symbols represent species from the genus indicated in the legend (Johnston, 1986).

dynamics. This information, coupled with microcosm and mesocosm experiments (Grassle and Grassle, 1984), could provide a valuable tool for assessing pollutant effects and assisting in the development of realistic ecological models that can be used to assess the impact of pollution to population and community dynamics.

RECOMMENDATIONS

Appendix A contains a table of preferred species for conducting aquatic toxicity testing. The table is arranged by taxonomic groups and includes marine bacteria, phytoplankton, crustaceans, molluscs, polychaete worms, and fish. The table provides information on the type of test performed, the parameter measured, the life history stages evaluated, and references to pertinent literature. As a ready reference of commonly used toxicity testing methodologies, this table will be useful to site managers who must evaluate the potential toxicological impact of sites at their facility (Johnston and Lapota, 1989).

The database of toxicity assessment methods contained in Appendix A should be used as a starting point in choosing the most applicable test for evaluating the impact of Navy hazardous waste disposal sites on aquatic environments. Some of the bioassays and indicator methods are fairly simple while others require extensive laboratory support. The choice of method should be driven by the extent of the problem at the site.

It is very important that the toxicological assessment information is integrated into the Remedial Investigation and Feasibility Study (RI/FS) process. One way to

effectively factor the toxicology data into the remediation process is illustrated in figure 16. The figure shows the RI/FS process actually consists of two parallel studies. One study is performed by the on-site contractor and focuses on characterizing contamination at the disposal site and potential migration to ground water. The second study is a

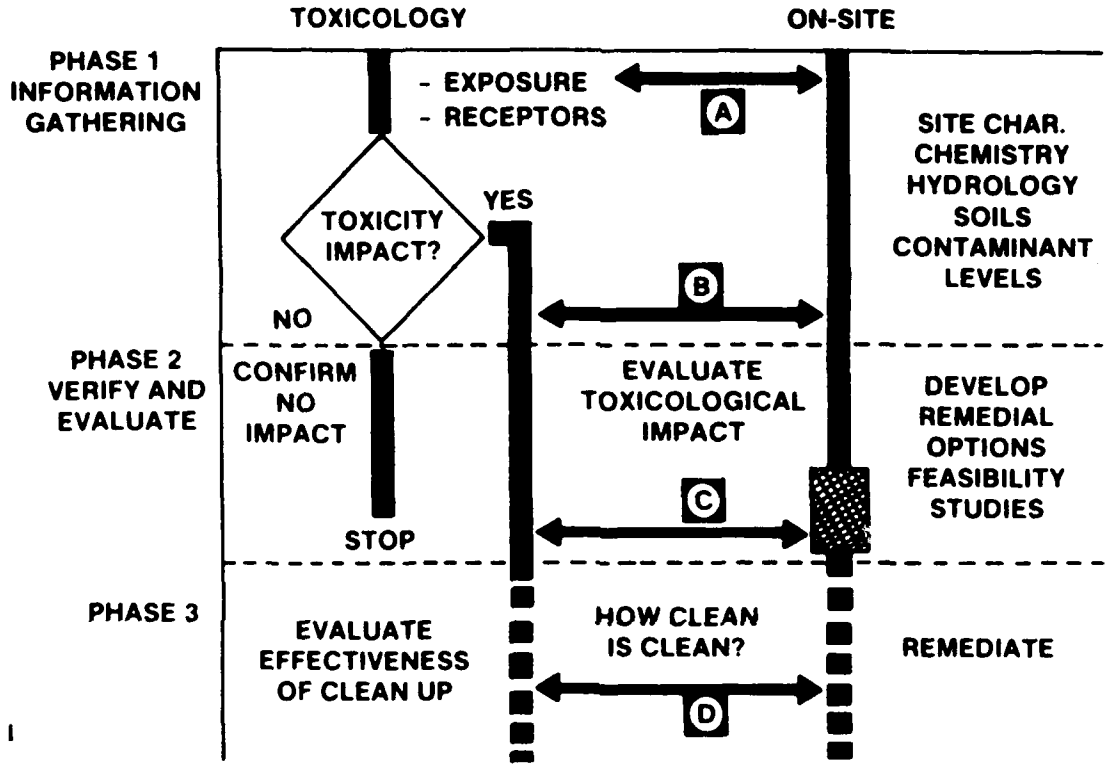

Figure 16. Integration of toxicological assessment information into the Remedial Investigation and Feasibility Study Process (Johnston and Lapota, 1989).

toxicological investigation which will focus on assessing whether pollutants at the site are impacting the surrounding environment and biota. These investigations may need to be performed by two separate contractors or agencies because the tasks involved require different capabilities. For example, a contractor who is skilled in installing ground water wells and conducting groundwater monitoring may not have the capability to perform toxicity tests. The toxicity tests maybe more appropriately performed by a local university or agency with demonstrated expertise in toxicological evaluations. Together these studies will meet the requirements defined by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) for the RI/FS process. These studies will also provide the data and information necessary to support risk management decisions and selection of the most effective remedial option.

Information transfer or feedback between the on-site investigation and the toxicology study is required to assure the two studies are complementary, comparable, and capable of achieving the desired objectives. Figure 16 shows the two concurrent studies are broken up into three phases with specific objectives for each phase. The double-headed arrows represent information transfer or feedback between the separate studies. Phase One represents information gathering. The objective of the on-site

investigation during Phase One is to define the site conditions and contamination levels, while the objective of the toxicology study is to determine if there is evidence of pollution impact to the surrounding environment.

There are two critical interaction points during the information gathering phase. The first (shown as A in figure 16) is to conduct an intercalibration of analytical methods to be used in the investigations. The basic goal of any QA/QC plan is to document that the numbers reported are reliable and scientifically valid. The on-site contractor will be using contract laboratory protocol (CLP) recommended by EPA Superfund guidance. These methods are effective for use at the site because they are designed to determine levels of contamination in the soil and groundwater matrices. The analytical objective of the toxicology study requires methods capable of achieving low (trace) level detection limits as well as being amenable for analysis in seawater, sediment, and tissue matrices. Consequently, an intercalibration between the two studies must be accomplished to provide a measure of comparability between the data sets.

The information transfer (shown by B in figure 16) provides feedback whenever significant information is developed that would benefit the other concurrent investigation. For example, if the toxicology data showed high levels of pesticide in clam tissues, this information would be needed by the on-site contractor to adequately characterize the pesticide source and distribution at the site. Similarly, if the on-site contractor's data showed excessive levels of cadmium, the toxicology study would need this information to accurately assess the toxicological response to cadmium. At the completion of Phase One, the on-site contractor will provide an accurate description of the site conditions and the toxicology study will provide a determination of environmental risk.

Phase Two objectives for the on-site contractor are to develop remedial options and conduct feasibility/treatability studies. Phase Two objectives for the toxicology study are to either verify the lack of adverse environmental impact or to evaluate the nature and extent of contaminant impact on the ecosystem. If there is no significant toxicological impact, the on-site contractor's chief concern is site closure. At this point, the toxicology study will develop a long-term monitoring strategy to assure continuous verification of environmentally safe levels. This information will be extremely useful for developing the risk management plan for the site. If there is a significant toxicological impact, then information developed by the toxicology study will be useful for evaluating potential remedial options and assessing the effectiveness of feasibility studies being developed by the on-site contractor. This interaction is shown as C in figure 16.

If remediation is necessary, Phase Three will be initiated. The toxicology study will be focused on evaluating the effectiveness of the remediation implemented by the on-site contractor. The interaction shown as D in figure 16 is necessary to determine "How Clean is Clean?" and when remediation should be terminated. The process described above is currently being put into practice for the risk assessment pilot study being conducted at Naval Construction Battalion Center, Davisville, RI, and for the toxicology demonstration project at NAS Whidbey Island, WA (Johnston *et al.*, 1988).

A combination of single and multispecies test procedures carried out in the laboratory and at the specific site are required to adequately assess the impact of contaminants on the surrounding ecosystem. Laboratory studies are useful for identifying the range or limits for impact which can be compared to field data. Multispecies testing will provide a range of sensitivities. The Navy should use existing data as a starting point for determining the optimum suite of tests that would be most applicable for detecting hazardous waste impact at a particular site. Assuming the species selected

accurately reflect the overall ecosystem response, it maybe practical to use only a few species for routine monitoring after test results are compared.

The benefit of conducting the toxicological assessment is evident in the information provided for the RI/FS. Not only will the toxicology data provide direct evidence of any impact, it will also help set levels that are deemed environmentally safe and acceptable. This information is invaluable in selecting optimal remedial options and managing the risks associated with hazardous waste disposal sites.

GLOSSARY

ACUTE TOXICITY = the ability of a substance to produce systematic damage in an organism from a single exposure, usually of short duration.

BIOLUMINESCENCE = the ability to produce light is a property of a number of marine living organisms including some bacteria, fungi, algae (dinoflagellates), fireflies, jellyfish, and crustaceans. The mechanism of light production involves energy transfer and production of an excited state in a molecule that emits light upon returning to the ground state.

CHEMOSTAT = a continuous flow-through system designed to produce steady-state conditions because the inflow and outflow rates are equal. Dilution rates for chemostats are determined by the flow rates and volume of the culture vessel. Growth is controlled by the concentration of a limiting nutrient.

CHRONIC TOXICITY = the ability of a substance to produce systematic damage in an organism from long term, usually low-level exposure.

COSMOPOLITAN = a species which is commonly found and widely distributed in many geographical regions.

CRUSTACEANS = a large subphylum of arthropods having typically biramous limbs and two pairs of antennae, such as crabs, lobsters, and shrimp.

EC_{50} = the effective concentration of a toxicant that inhibits 50 percent of a sublethal parameter of a test organism, such as growth or light output.

ECHINODERMS = a phylum of spiny-skinned marine animals such as starfish and sea urchins.

ECOSYSTEM = a natural unit of living and nonliving parts that interact to produce a stable system in which the exchange of materials between living and nonliving parts follows a circular path.

ECOTOXICITY = the study of the effects of released pollutants on the environment and the biota that inhabit it.

ED_{50} = the effective dose 50 or the median dose, i.e., the dose of a substance either beneficial or toxic required to result in a response in 50 percent of a test population.

EUTROPHICATION = nutrient enrichment of natural waters, usually from man-made sources, which frequently leads to excessive algal growth.

F_1 GENERATION = first filial generation or first produced generation resulting when genes from two different parents come to reside in a single cell. The filial generation will have a genotype reflective of the contributing parents but may resemble only one of the parents.

FIELD VALIDATION = a comparison of the overall results of a method, toxicity test, or model with field data in order to determine the degree of correspondence.

GAMETE = a reproductive cell such as an egg or sperm whose union in sexual reproduction initiates development of a new individual.

GAMMA FUNCTION = the ratio of the amount of light lost to the amount of light remaining; this function has been reported to be more precise in defining percent light decrease in the Microtox luminescent assay.

GASTRULA = early embryonic stage of an organism which follows the blastula; consists initially of two layers of cells, the ectoderm and the endoderm, and of two cavities, the blastocoele and the archenteron formed by invagination.

GROWTH INHIBITION INDEX = the average reduction in cell number exposed to a toxicant during a test period as compared to the control.

HALIOTIID = a gastropod mollusc in the order Archaeogastropoda; a primitive form. Shell is coiled, many marine species such as the abalone *Haliotis*.

HERBIVOROUS = feeding on plants.

HYDROLASE = breakdown of a polymer into smaller units, usually monomers, by addition of water; digestion.

HYPERACTIVITY = a state of altered activity; abnormally increased activity; ability to react with characteristic symptoms to the presence of certain substances.

INHIBITORY = prevention of growth or function.

IN VIVO = in the body, in a living organism.

LC_{50} = lethal concentration 50 is the concentration of a chemical in air or water that causes death to 50 percent of the test organisms.

LOG PHASE = the growth of a population of cells generally occurs at an exponential rate. After a cell divides to form two daughter cells each daughter cell in turn divides and produces two new cells, so at each division period the population doubles. Population growth of this type is called exponential growth or log phase growth and is characteristic of unicellular organisms.

LUCIFERASE = an enzyme which is present in luminous plants and animal species and which, acting upon luciferin, produce luminosity.

LYSOZYMES = a basic bacteriolytic protein that functions as a mucolytic enzyme.

MICROCOSM = a community or other unity that is an epitome of a larger unity.

MOLLUSCS = a phylum of soft-bodied animals usually in shells, including snails and clams.

MORIBUND = being in the state of dying: approaching death.

MOTILITY = property of movement of a cell under its own power.

NEOPLASMS = a new growth of tissue serving no physiological function: tumor.

NONTHECATE = without having a case, covering, or sheath.

PELAGIC ZONE = comprises the waters of the open ocean. Because of its volume, expanse, and the density of its population, it forms the major oceanic environment.

PERISTALTIC = a pump in which fluid is forced along by waves of contraction produced mechanically on flexible tubing, thus preventing contact with and contamination from the pump parts.

PHOTODETECTOR = a sensor which measures light.

POLYCHAETES = a class of annelid (segmented) worms with parapodia (appendages) bearing numerous bristles.

PRIMARY PRODUCERS = plants found both on land in the sea which transform solar energy into chemical energy. For our purposes phytoplankton species, which are microscopic in size, are the primary producers in the sea. Total primary production is usually determined by available light and nutrients which are critical for plant growth.

SEDENTARY = referring to animals that move about little or are permanently attached; not migratory.

SCOPE FOR GROWTH (SFG) INDEX = a measure of the energy available to an organism for production, both somatic and reproductive, after accounting for routine metabolic costs. The SFG value represents the instantaneous assessment of energy balance in an organism for that set of environmental conditions under which it is measured.

SOMATIC = referring to the body wall of an organism such as body wall muscles.

STATIONARY PHASE = period during the growth cycle of a population in which growth ceases.

STIMULATORY = an agent (as an environmental change) that directly influences the activity of living protoplasm.

TOXICANT = environmental pollutant.

TOXIN = plant or animal secreted poison.

TROPHIC LEVELS = components of the food web such as the grazing food chain whereby, for example, organisms of a natural community whose food is obtained from plants by the same number of steps are said to belong to the same trophic level. Green plants (the producer level) occupy the first trophic level, plant eaters the second level (the primary consumer level), carnivores, which eat the herbivores, the third level (the secondary consumer level), and secondary carnivores the fourth level (the tertiary consumer level). It should be emphasized that trophic classification is one of function and not of species as such.

TURBIDOSTAT = a method of estimating bacterial growth or populations by measurement of the degree of opacity (or turbidity) of the suspension of cells. A steady state regulation is accomplished by a sensor placed within the cultured community that responds to the density of the organisms.

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APPENDIX A
PREFERENCE LIST OF SPECIES FOR TOXICITY TESTING AT NAVY HAZARDOUS WASTE DISPOSAL SITE NEAR AQUATIC ENVIRONMENTS

Preference List of Species for Toxicity Testing at Navy Sites

Group/Species	Parameter Measured	Life History	Reference
Marine Bacteria:			
Photobacterium phosphoreum	Luminescence (Microtox)™	Cell death	Bulich *et al.*, 1981
Marine Phytoplankton:			
Dunaliella tertiolecta	Batch Method – population growth	Cell growth	Jenson *et al.*, 1976; Fisher & Frood, 1981
Thalassiosira psuedonana	Batch Method – population growth	Cell growth	Jenson *et al.*, 1976; Fisher & Frood, 1981
Skeletonema costatum	Batch Method – population growth	Cell growth	Jenson *et al.*, 1976; Fisher & Frood, 1981
Phaedoctylum tricornutum	Batch Method – population growth	Cell growth	Jenson *et al.*, 1976; Fisher & Frood, 1981
Dinoflagellates:			
Prorocentrum micans	Population growth	Cell growth	Prevot & Soyer, 1978
Gymnodinium breve	Population growth	Cell growth	Kutt & Martin, 1974
Gymnodinium splendens	Population growth-chlorophyll fluorescence	Cell growth	Krett, 1979
Gonyaulax taurarensis	Population growth	Cell growth	Anderson & Morel, 1978
Pyrocystis spp.	Luminescence	Cell growth	Hannan *et al.*, 1986

Preference List of Species for Toxicity Testing at Navy Sites (contd)

Group/Species	Parameter Measured	Life History	Reference
Marine Crustaceans:			
Artemia (Brine shrimp)	Lethality	Naupliar larvae	Vanhaecke & Persoone, 1984
Acartia tonsa (Copepod)	Acute & chronic	Multi-generation chronic exposures	Heinle & Beaven, 1980
Balanus spp. (Barnacles)	Survival, molting, behavior & larval settlement	Naupliar larvae	Lang, *et al.*, 1980
Mysidopsis bahia (Mysid shrimp)	Acute, chronic, growth rate, mortality, fecundity	Juveniles, EPA-routine; ASTM protocol	Nimmo & Hamaker, 1982 J. Gentile *et al.*, 1983
Mysidopsis bigelowi (Mysid shrimp)	Acute, chronic effects, survival & reproductive success	Chronic life cycle studies	S. Gentile *et al.*, 1882
Acanthomysis sculpta (Mysid shrimp)	Acute, chronic studies, mortality, growth & reproduction	Adults, juveniles	Davidson *et al.*, 1986
Rhepoxynius abronius (Burrowing amphipod)	Acute-lethality 10 day exposure	Adults	Swartz *et al.*, 1985
Palaemonetes spp. (Grass shrimp)	Static-flow, molting	Larval stages	Buikema *et al.*, 1980
Homarus americanus (American lobster)	Mortality	Larval stages (1-3)	Wells & Sprague, 1976

Preference List of Species for Toxicity Testing at Navy Sites (contd)

Group/Species	Parameter Measured	Life History	Reference
Rhithropanopeus harvisii (Mud crab)	Acute-chronic sublethal (growth, development, molting, respiratory physiology, metabolism, behavior)	Larval stages	Bookhout & Costlow, 1974
Marine Mollusks:			
Crassostrea virginica (American oyster)	Abnormal shell development, mortality	Embryo-larval test, 0-48 hrs old	ASTM, 1980
Crassostrea gigas (Pacific oyster)	Abnormal shell development, mortality	Embryo-larval test, 0-48 hrs old	Woelke, 1972; ASTM, 1980
Mytilus edulis (Blue mussel)	Sublethal, Scope for Growth measurements; growth rates	Juveniles, adults. shell growth	Phelps *et al.*, 1983; Valkirs *et al.*, 1985; Salazar & Salazar, 1987
Mercenaria mercenaria (Hard clam)	Sublethal, Scope for Growth measurements; growth rates	Juveniles, adults, shell growth	ASTM, 1980
Crepidula fornicata (Slipper shell)	Survival & growth, fecundity, viability of succeeding generations	Larvae, adults	Calabrese *et al.*, 1979 Nelson *et al.*, 1983
Haliotis rufescens (Red abalone)	Abnormal larval shell development	Veliger stage	Martin *et al.*, 1986

Preference List of Species for Toxicity Testing at Navy Sites (contd)

Group/Species	Parameter Measured	Life History	Reference
Echinoderms:			
Strongylocentrotus drobachiensis (Sea urchin)	Fertilization rate	Gametes, eggs & sperm	Hagstron & Lonning, 1977
Strogylocentrotus purpuratus (Sea urchin)	Fertilization success	Sperm-toxicity test	Dinnel *et al.*, 1982
Arbacia punctulata (Sea urchin)	Hatching success, gastrula stage, 48-hr pluteus stage;	Gametes & embryo	Pagano *et al.*, 1982
	Fertilization success, incidence of developmental abnormalities	Gamete develcpmental stages	Brown & Greenwood, 1978; Oshida *et al.*, 1981
	Fertilization success, abnormal developmental stages	Fertilized cell, first cleavage, polyspermic cleavage	Kobayshi, 1971, 1977
Polychaetous Annelids:			
Neanthes arenaceodenta (Marine worm)	96-hr LC-50; 28-day reproduction test	Adult	Oshida & Word, 1982; Pesch & Hoffman, 1983
Capitella capitata (Marine worm)	96-hr LC-50	Adult	Reish, *et al.*, 1983
Ophryotrocha diadema (Marine worm)	96-hr LC-50	Adult	Reish, 1980

Preference List of Species for Toxicity Testing at Navy Sites (contd)

Group/Species	Parameter Measured	Life History	Reference
Fish:			
Fundulus heteroclitus (Killifish)	0-96 hr, 4-28 days, >28 days lethality	Adult	EPA, 1978
Oncorhynchus kisutch (Coho salmon)	Salt & fresh water mortality	Adult & juveniles	Chapman & Stevens, 1978; Sugatt, 1980
Gasterosteus aculeatus (Three spine stickelback)	Lethality	Adult	EPA, 1978; Katz & Chadwick, 1961
Pleuronectes platessa (Plaice)	Fertilization success	Eggs, Larvae	Lonning, 1977; Lonning & Hagstrom, 1976; Wilson, 1976
Platichthys flesus (Flounder)	Sublethal physiological effects, hyperactivity	Adult	Bengtsson & Larsson, 1981; Haux *et al.*, 1982
	Altered carbohydrate metabolism	Adult	Larsson & Haux, 1982
	Fertilization & development	Adult	Lonning & Hagstrom, 1976